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# Statistical Assessment of the Age–Length Key

DANIEL K. KIMURA

*Washington Department of Fisheries, Olympia, Wash. 98504, USA*

KIMURA, D. K. 1977. Statistical assessment of the age–length key. J. Fish. Res. Board Can. 34: 317–324.

Since 1934 when Fridriksson originated the age–length key, it has been widely used by fisheries biologists to estimate age distributions of populations. In recent years, there has been a general recognition that often the key has little value, or even worse, gives biased results. The analysis presented here indicates why the age–length key is so susceptible to bias. More importantly, a criterion is presented for determining whether the age–length key should be used in a particular situation. If the key is to be used, results from examples indicate that random age subsamples (i.e. the number of specimens aged from each length category proportional to the number in each length category) are superior to fixed age subsamples (i.e. a constant number of specimens aged from each length category). Generally, small increases in the age sample will likely increase the accuracy of an age-distribution determination more effectively than relatively large increases in the length sample.

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Introduite en 1934 par Fridriksson, la clé âge–longueur a été depuis largement utilisée par les biologistes des pêches pour estimer la distribution des âges dans une population. Depuis quelques années toutefois, on admet généralement que la clé a souvent peu de valeur ou, ce qui est pire, donne des résultats biaisés. L'analyse que nous présentons ici explique pourquoi la clé âge–longueur est si susceptible de biais. Ce qui est plus important, nous donnons un critère permettant de décider si la clé âge–longueur doit être utilisée dans une situation particulière. Dans les cas où la clé doit être utilisée, des exemples indiquent que les sous-échantillons d'âges pris au hasard (i.e. le nombre de spécimens dont l'âge est déterminé dans chaque catégorie de longueur étant proportionnel au nombre de poissons dans chaque catégorie de longueur) sont supérieurs à des sous-échantillons d'âges fixes (i.e. un nombre uniforme de spécimens dont l'âge est déterminé dans chaque catégorie de longueur). Généralement, des petites augmentations dans l'échantillon d'âges résulteront probablement en une détermination plus précise de la répartition des âges que ne le feraient des augmentations relativement grandes de l'échantillon de longueurs.

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ONE of the fundamental problems of fisheries biologists is to estimate the age distribution of a population. Sampling for ages may be accomplished by either taking a simple random sample, or by using Fridriksson's (1934) age–length key. The biologist must decide if the gains made by using the age–length key justify its use. There appears to be little in the literature to help him make this decision. In this paper, a criterion is presented for determining whether the key should be used in a particular situation.

If the age–length key is to be used, the biologist must decide whether the age subsample should be random (i.e. the number of specimens aged from each length category proportional to the number in each length category) or fixed (i.e. a constant number of specimens aged from each length category). In the literature, one can find proponents of random and fixed age subsamples. Ketchen (1949), in an early paper, felt that fixed age subsamples were superior. Southward (1976) found that random age subsamples were more efficient. The analyses and examples presented in the following sections show that from a statistical point of view random age subsamples are more efficient.

## Age–Length Key

Suppose the proportions of a population of age $i = 1, \ldots, n_a$ are $p_1, \ldots, p_{n_a}$. Suppose also that the distribution of lengths at each age is given by $q_{ij}$, $j = 1, \ldots, n_l$.[1] Here,

$$\sum_{j=1}^{n_l} q_{ij} = 1$$

for all $i$. The proportion of length $j$ is then

$$l_j = \sum_{i=1}^{n_a} p_i q_{ij}.$$

Of the $j$ length fish, the proportion of fish of age $i$ is $q_{ij}' = (p_i q_{ij})/l_j$. Therefore,

$$\sum_{i=1}^{n_a} q_{ij}' = 1$$

for all $j$. The quantities directly estimable from sample data are $l_j$ and $q_{ij}'$. If a random sample of $N_l$ length frequencies is taken and $n_j$ are of length $j$, and if $n_j^*$ of the $n_j$ fish are aged, of which $m_{ij}$ are of age $i$, then $\hat{l}_j = n_j/N_l$ and $\hat{q}_{ij}' = m_{ij}/n_j^*$ are unbiased estimates of $l_j$ and $q_{ij}'$. Since

$$p_i = \sum_{j=1}^{n_l} l_j q_{ij}',$$

it follows that

$$\hat{p}_i = \sum_{j=1}^{n_l} \hat{l}_j \hat{q}_{ij}'$$

provides an unbiased estimate of $p_i$. This is the usual application of the age–length key. It should be noted that $q_{ij}'$ is dependent on the $p_i$'s. This means that the age–length key will give biased results if applied to a population where the age composition differs from that of the population from which the age–length key was drawn.

The value of this key can be assessed directly by calculating the variance of the estimates of the various $p_i$'s. Any gains from increasing the length-frequency sample should be reflected in the variance of the $\hat{p}_i$'s. Since we are concerned with the overall variance of the $\hat{p}_i$'s, the total variance

$$\text{Vartot} = \text{E} \sum_{i=1}^{n_a} (\hat{p}_i - p_i)^2 = \sum_{i=1}^{n_a} \text{var}\,(\hat{p}_i)$$

[1]It should be noted that $n_a$ and $n_l$ actually refer to the number of categories. The ages and lengths are assumed to be coded so that the smallest values are one.

seems an appropriate error index. Using this error index, the efficiency of the age–length key may be assessed, as compared with increasing the age sample. Also, it may be determined whether the age subsample should be random or fixed.

## Derivation of the Variance of the $\hat{p}_i$'s

The variance for the $\hat{p}_i$'s estimated from the age–length key may be derived by straightforward methods. However, for simplification, the following assumptions are made concerning $n_j^*$, the subsample from length category $j$ which is aged. For the case of fixed age subsample sizes, the variance is conditioned on $n_j^* = n_s/n_l$, where $n_s$ is the total sample to be aged and $n_l$ is the number of length categories. The weakness of this approach is that $n_j$, the number of specimens of length $j$, may be actually less than $n_j^*$. When $N_l$ is large compared with $n_s$, this should not be a problem. For the case of random age subsamples, it is assumed that $n_j^* = sn_j$, where $s = n_s/N_l$. Hence, this variance is not conditioned on $n_j^*$.

The variance of $\hat{p}_i$,

$$\begin{aligned}\mathrm{Var}(\hat{p}_i) &= \mathrm{E}(\hat{p}_i - p_i)^2 \\ &= \mathrm{E}\sum_{j=1}^{n_l}(\hat{l}_j\hat{q}'_{ij} - l_jq'_{ij})\sum_{j'=1}^{n_l}(\hat{l}_{j'}\hat{q}'_{ij'} - l_{j'}q'_{ij'}) \\ &= \sum_{j=1}^{n_l}\sum_{j'=1}^{n_l}\mathrm{E}(\hat{l}_j\hat{q}'_{ij}\hat{l}_{j'}\hat{q}'_{ij'} - l_jq'_{ij}l_{j'}q'_{ij'}) \qquad (1)\end{aligned}$$

The expectations can most easily be evaluated by breaking them into the cases $j = j'$ and $j \neq j'$. When $j = j'$ the expectation gives a different result under fixed and random subsampling.

Case 1a. $j = j'$ subsample fixed

$$\begin{aligned}\mathrm{E}(\hat{l}_j\hat{q}'_{ij}\hat{l}_j\hat{q}'_{ij}) &= \mathrm{E}\left(\frac{n_j}{N_l}\frac{m_{ij}}{n_j^*}\frac{n_j}{N_l}\frac{m_{ij}}{n_j^*}\right) \\ &= \mathop{\mathrm{E}}_{n_j}\frac{n_j}{N_l}\frac{n_j}{N_l}\left(\frac{q'_{ij}(1-q'_{ij})}{n_j^*} + q'^2_{ij}\right) \\ &= \left[\frac{l_j(1-l_j)}{N_l} + l_j^2\right]\left[\frac{q'_{ij}(1-q'_{ij})}{n_j^*} + q'^2_{ij}\right]\end{aligned}$$

Case 1b. $j = j'$ subsample random

$$\begin{aligned}\mathrm{E}(\hat{l}_j\hat{q}'_{ij}\hat{l}_j\hat{q}'_{ij}) &= \mathrm{E}\left(\frac{n_j}{N_l}\frac{m_{ij}}{sn_j}\frac{n_j}{N_l}\frac{m_{ij}}{sn_j}\right) \\ &= \mathop{\mathrm{E}}_{n_j}\frac{n_j}{N_l}\frac{n_j}{N_l}\left[\frac{q'_{ij}(1-q'_{ij})}{sn_j} + q'^2_{ij}\right] \\ &= \frac{l_jq'_{ij}(1-q'_{ij})}{n_s} + q'^2_{ij}\left[\frac{l_j(1-l_j)}{N_l} + l_j^2\right]\end{aligned}$$

Case 2. $j \neq j'$ subsample fixed or random

$$\begin{aligned}\mathrm{E}(\hat{l}_j\hat{q}'_{ij}\hat{l}_{j'}\hat{q}'_{ij'}) &= \mathrm{E}\left(\frac{n_j}{N_l}\frac{m_{ij}}{n_j^*}\frac{n_{j'}}{N_l}\frac{m_{ij'}}{n_{j'}^*}\right) \\ &= \mathop{\mathrm{E}}_{n_j,n_{j'}}\left[\frac{n_j}{N_l}\frac{n_{j'}}{N_l}q'_{ij}q'_{ij'}\right] \\ &= q'_{ij}q'_{ij'}\left[\frac{-l_jl_{j'}}{N_l} + l_jl_{j'}\right]\end{aligned}$$

Substituting the appropriate expectations back into (1), and simplifying, yields the following variance formulas:

a) $\text{Var}(\hat{p}_i) = \dfrac{p_i(1-p_i)}{n_s}$ (random sample)

ages only

b) $\text{Var}(\hat{p}_i) = \sum_{j=1}^{n_l} \left[ \dfrac{l_j(1-l_j)}{N_l} \dfrac{q'_{ij}(1-q'_{ij})}{n_j^*} + \dfrac{l_j^2 q'_{ij}(1-q'_{ij})}{n_j^*} + \dfrac{l_j q'^2_{ij}}{N_l} \right] - \dfrac{p_i^2}{N_l}$

age subsample fixed

c) $\text{Var}(\hat{p}_i) = \sum_{j=1}^{n_l} \left[ \dfrac{l_j q'_{ij}(1-q'_{ij})}{n_s} + \dfrac{l_j q'^2_{ij}}{N_l} \right] - \dfrac{p_i^2}{N_l}$

age subsample random

Note that $\text{Var}(\hat{p}_i)$ and $\text{Vartot} = \sum_{i=1}^{n_a} \text{Var}(\hat{p}_i)$ have the following forms:

a) $\text{Vartot} = \dfrac{a_1}{n_s}$

ages only

b) $\text{Vartot} = \dfrac{b_1}{N_l n_j^*} + \dfrac{b_2}{n_j^*} + \dfrac{b_3}{N_l}$

age subsample fixed

c) $\text{Vartot} = \dfrac{c_1}{n_s} + \dfrac{c_2}{N_l}$

age subsample random

The form of these Vartot expressions allow us to easily calculate the asymptotic value of Vartot as $N_l$ goes to infinity. This value of Vartot would be that given by an age–length key when an infinite length sample was taken. We can then calculate the asymptotic percentage reduction in Vartot (ages only), which would then represent an upper bound to the value of using the key. An important property of this percentage reduction is that it is independent of the actual value of $n_s$. In other words, the gain in using the age–length key is the same when $n_s = 100$, or $n_s = 1000$, when measured in this way.

The asymptotic variances can be written as

$$\lim_{N_l \to \infty} \begin{matrix} \text{Vartot} \\ \text{age subsample} \\ \text{fixed} \end{matrix} = \frac{b_2}{n_j^*} = n_l \frac{b_2}{n_s}$$

and

$$\lim_{N_l \to \infty} \begin{matrix} \text{Vartot} \\ \text{age subsample} \\ \text{random} \end{matrix} = \frac{c_1}{n_s}$$

The percentage reduction in Vartot (ages only) that can be achieved when age subsamples are fixed is then

$$\gamma_F = \frac{\dfrac{a_1}{n_s} - n_l \dfrac{b_2}{n_s}}{\dfrac{a_1}{n_s}} \times 100 = \frac{a_1 - n_l b_2}{a_1} \times 100$$

Since $p_i = \sum_{j=1}^{n_l} l_j q'_{ij}$ implies $a_1 = c_1 + c_2$, it may be deduced that $\begin{matrix}\text{Vartot}\\ \text{ages}\\ \text{only}\end{matrix} = \begin{matrix}\text{Vartot}\\ \text{age subsample}\\ \text{random}\end{matrix}$

when $N_l = n_s$ (i.e. when the length observations are taken from specimens that are aged). Therefore, the percentage reduction in Vartot (ages only) that can be achieved when age subsamples are random is

$$\gamma_R = \frac{\frac{a_1}{n_s} - \frac{c_1}{n_s}}{\frac{a_1}{n_s}} \times 100 = \frac{c_2}{a_1} \times 100$$

The age–length key, when age subsamples are random, cannot do worse than a random sample of ages without lengths. However, when age subsamples are fixed, the age–length key may do worse than a random sample of ages even if an infinite length sample were taken. In the following section, an example is given in which this is the case.

## Examples

As examples of how the preceding analyses can be used, age–length data were compiled from biological market samples for the following two species:

a) Species: Pacific ocean perch
(*Sebastes alutus*)
Location: Queen Charlotte Sound
Time: May–June–July 1975
Sex: male
Ages: 9–20 yr
Lengths: 32–43 cm

b) Species: Pacific cod
(*Gadus macrocephalus*)
Location: miscellaneous
Time: February–March 1974
Sex: male
Ages: 3–5 yr
Lengths: 53–77 cm

From the data presented in Tables 1 and 2, estimates of $p_i$ and $q_{ij}$ were made which were then assumed to be the true parameters of the population. The coefficients for Vartot were then calculated from the formulas given in the preceding section. The calculated coefficients are as follows:

| Pacific ocean perch | Pacific cod |
|---|---|
| $a_1 = .85135$ | $a_1 = .54270$ |
| $b_1 = .66564$ | $b_1 = .25866$ |
| $b_2 = .09106$ | $b_2 = .01798$ |
| $b_3 = .09465$ | $b_3 = .26605$ |
| $c_1 = .75670$ | $c_1 = .27665$ |
| $c_2 = .09465$ | $c_2 = .26605$ |
| $\gamma_F = -28\%$ | $\gamma_F = +17\%$ |
| $\gamma_R = +11\%$ | $\gamma_R = +49\%$ |

From the $\gamma_F$ value, it is seen that for Pacific ocean perch, Vartot (ages only) increases 28% when an age–length key is used with fixed age subsamples. This means that the age determination is worse

TABLE 1. Age–length data for Pacific ocean perch (*Sebastes alutus*).

| Length (*cm*) | Age (*yr*) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 32 | 10 | 5 | | | | | | | | | | |
| 33 | 15 | 13 | 13 | 1 | | | | | | | | |
| 34 | 14 | 27 | 27 | 19 | 4 | 1 | | | | | | |
| 35 | 26 | 36 | 59 | 32 | 3 | 2 | | | | | | |
| 36 | 5 | 48 | 74 | 67 | 18 | 3 | 1 | | | | | |
| 37 | 3 | 23 | 51 | 37 | 20 | 7 | | 1 | | | | |
| 38 | | 6 | 23 | 40 | 27 | 12 | 2 | | 2 | | | |
| 39 | | | 5 | 10 | 13 | 23 | 9 | 6 | | 2 | | 1 |
| 40 | | | 1 | 1 | 12 | 13 | 14 | 18 | 6 | 2 | 1 | |
| 41 | | | | 1 | 5 | 9 | 11 | 18 | 11 | 3 | 6 | 1 |
| 42 | | | | | | 5 | 7 | 7 | 7 | 9 | 6 | 5 |
| 43 | | | | | | 1 | 2 | 1 | 2 | 1 | 1 | 4 |

TABLE 2. Age–length data for Pacific cod (*Gadus macrocephalus*).

| Length (*cm*) | Age (*yr*) | | |
|---|---|---|---|
| | 3 | 4 | 5 |
| 53 | 5 | | |
| 54 | 3 | | |
| 55 | 6 | | |
| 56 | 13 | 3 | |
| 57 | 11 | | |
| 58 | 14 | 3 | |
| 59 | 18 | 4 | |
| 60 | 20 | 2 | |
| 61 | 18 | 2 | |
| 62 | 15 | 10 | |
| 63 | 13 | 1 | |
| 64 | 8 | 9 | 1 |
| 65 | 6 | 14 | |
| 66 | 3 | 13 | |
| 67 | 1 | 13 | |
| 68 | 1 | 8 | 2 |
| 69 | | 13 | 4 |
| 70 | 1 | 7 | |
| 71 | | 4 | 2 |
| 72 | | | 2 |
| 73 | | | 2 |
| 74 | | 1 | |
| 75 | | | 2 |
| 76 | | | 1 |
| 77 | | | 2 |

than a simple random sample of ages even if an infinite length sample is taken. From the $\gamma_R$ value, it is seen that Vartot (ages only) decreases 11% when an age–length key is used with random age subsamples. The age-length key is apparently of marginal value in this case.

For Pacific cod, $\gamma_F = 17\%$ and $\gamma_R = 49\%$. The age–length key may be of significant value. Again, random age subsamples are favored over fixed age subsamples. Tables 3 and 4 give values of Vartot at various sampling levels. For consistency, sample sizes were rounded to integers. From these tables, it appears that random samples are generally favored over fixed samples. For populations

TABLE 3. Values of Vartot for Pacific ocean perch. F and R refer to fixed and random age subsamples.

| Aged sample | Length sample = aged sample | Total length sample | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | | 200 | | 300 | | 400 | | 500 | | 1000 | |
| | | F | R | F | R | F | R | F | R | F | R | F | R |
| 50 | .01703 | .02538 | .01608 | .02407 | .01561 | .02364 | .01545 | .02342 | .01537 | .02329 | .01532 | .02303 | .01523 |
| 100 | .00851 | .01316 | .00851 | .01227 | .00804 | .01198 | .00788 | .01183 | .00780 | .01174 | .00776 | .01156 | .00766 |
| 150 | .00568 | | | .00773 | .00552 | .00749 | .00536 | .00737 | .00528 | .00730 | .00523 | .00715 | .00514 |
| 200 | .00426 | | | .00603 | .00426 | .00580 | .00410 | .00569 | .00402 | .00562 | .00397 | .00549 | .00388 |
| 250 | .00341 | | | | | .00476 | .00334 | .00465 | .00326 | .00459 | .00322 | .00446 | .00312 |
| 300 | .00284 | | | | | .00405 | .00284 | .00395 | .00276 | .00389 | .00271 | .00376 | .00262 |
| 350 | .00243 | | | | | | | .00343 | .00240 | .00338 | .00235 | .00326 | .00226 |
| 400 | .00213 | | | | | | | .00305 | .00213 | .00299 | .00208 | .00287 | .00199 |
| 450 | .00189 | | | | | | | | | .00262 | .00187 | .00251 | .00178 |
| 500 | .00170 | | | | | | | | | .00239 | .00170 | .00228 | .00161 |

TABLE 4. Values of Vartot for Pacific cod. F and R refer to fixed and random age subsamples.

| Aged sample | Length sample = aged sample | Total length sample | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | | 200 | | 300 | | 400 | | 500 | | 1000 | |
| | | F | R | F | R | F | R | F | R | F | R | F | R |
| 50 | .01085 | .01295 | .00819 | .01097 | .00686 | .01031 | .00642 | .00998 | .00620 | .00978 | .00607 | .00939 | .00580 |
| 100 | .00543 | .00780 | .00543 | .00615 | .00410 | .00560 | .00365 | .00532 | .00343 | .00516 | .00330 | .00483 | .00303 |
| 150 | .00362 | | | .00454 | .00317 | .00403 | .00273 | .00377 | .00251 | .00362 | .00238 | .00331 | .00211 |
| 200 | .00271 | | | .00374 | .00271 | .00324 | .00227 | .00299 | .00205 | .00284 | .00192 | .00255 | .00165 |
| 250 | .00217 | | | | | .00277 | .00199 | .00253 | .00177 | .00238 | .00164 | .00209 | .00137 |
| 300 | .00181 | | | | | .00246 | .00181 | .00222 | .00159 | .00207 | .00145 | .00179 | .00119 |
| 350 | .00155 | | | | | | | .00200 | .00146 | .00185 | .00132 | .00157 | .00106 |
| 400 | .00136 | | | | | | | .00183 | .00136 | .00169 | .00122 | .00141 | .00096 |
| 450 | .00121 | | | | | | | | | .00156 | .00115 | .00128 | .00088 |
| 500 | .00109 | | | | | | | | | .00146 | .00109 | .00118 | .00082 |

of different age structures, the analysis could change significantly. However, these changes can be examined by the method presented here.

In addition to the analysis presented here, the variance formulas can be used to examine each $\hat{p}_i$ on an individual basis. For example, the coefficient of variation of each $\hat{p}_i$ may be calculated. This type of analysis tends to be voluminous when many sampling levels are being examined.

Finally, the variance of the $\hat{p}_i$'s estimated from the actual use of an age–length key may be estimated by simply substituting the appropriate parameter estimates into the var($\hat{p}_i$) formula for the case where the age subsample is fixed. The derivation of this formula only required that the $n_j$*'s were fixed constants, not necessarily equal.

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# Host and Seasonal Associations of *Echinorhynchus salmonis* (Acanthocephala: Echinorhynchidae) in Lake Michigan Fishes

OMAR M. AMIN AND JANET M. BURROWS

*Science Division, University of Wisconsin-Parkside, Kenosha, Wis. 53140, USA*

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*Echinorhynchus salmonis* Müller was the dominant intestinal parasite infecting 14 species of southwestern Lake Michigan fishes. New host records are reported for chinook salmon, *Oncorhynchus tshawytscha*, and slimy sculpin, *Cottus cognatus*. Infections were heaviest in members of the Salmonidae. Host–parasite relationship in smelt was most closely analyzed. No pronounced seasonal periodicity in the rate and intensity of infection or worm maturation was observed. Recruitment and maturation occurred throughout the year but were, however, enhanced by colder and warmer temperatures, respectively. Sex ratio was nearest to even in winter. Fifty percent or more of worms were localized in the posterior-most region of host intestine and posterior migration with maturation was indicated. Heavier infections were noted in larger (older) fishes. Alternative routes of infection of the larger piscivorous salmonids via transfer hosts are suggested. Female hosts were more heavily infected than males. *Echinorhynchus salmonis* was not shown to seriously threaten its host's health or survival.

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*Echinorhynchus salmonis* Müller est le parasite intestinal dominant chez 14 espèces de poissons du sud-ouest du lac Michigan. Nous le signalons pour la première fois chez le saumon chinook (*Oncorhynchus tshawytscha*) et le chabot visqueux (*Cottus cognatus*). Les membres de la famille des Salmonidae sont ceux qui sont le plus fortement infestés. Nous avons examiné de plus près la relation hôte-parasite chez l'éperlan. Nous n'avons pas observé de périodicité saisonnière marquée dans le taux et l'intensité d'infestation, non plus que dans la maturation des vers. Le recrutement et la maturation se produisent à l'année longue, mais sont toutefois favorisés par de froides et de chaudes températures respectivement. La proportion des sexes se rapproche le plus de l'égalité en hiver. Cinquante pour cent ou plus des vers se rencontrent dans la région la plus arrière de l'intestin de l'hôte, et il y a indication d'une migration vers l'arrière avec la maturation. On note une infestation plus forte chez les grands (vieux) poissons. On croit qu'il existe des voies alternatives d'infestation des grands salmonidés piscivores via des hôtes intermédiaires. Les hôtes femelles sont plus lourdement infestés que les mâles. Il n'y a pas d'indication que *E. salmonis* menace sérieusement la santé ou la survie de son hôte.

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EIGHTEEN species of fishes were collected from southwestern Lake Michigan near the Wisconsin–Illinois state line during 1973–4. Two acanthocephalan and a number of other helminth species were recovered. The objective of this study was to explore the seasonal distribution and the ecology of the more common *Echinorhynchus salmonis* Müller, 1784, in its fish hosts. This acanthocephalan species is a common fish parasite in Europe and North America and is often reported from the Great Lakes region. This is the first complete seasonal investigation of *E. salmonis* infections in Lake Michigan fishes. Information on other parasites will be reported elsewhere.

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## Materials and Methods

Fishes were collected at three sites near the Wisconsin–Illinois state line between November 1973 and October 1974 by gillnetting and/or trawling about twice monthly (Fig. 1). A pilot study was undertaken in the same area during April 1973 from which recovered *E. salmonis* were incorrectly reported as

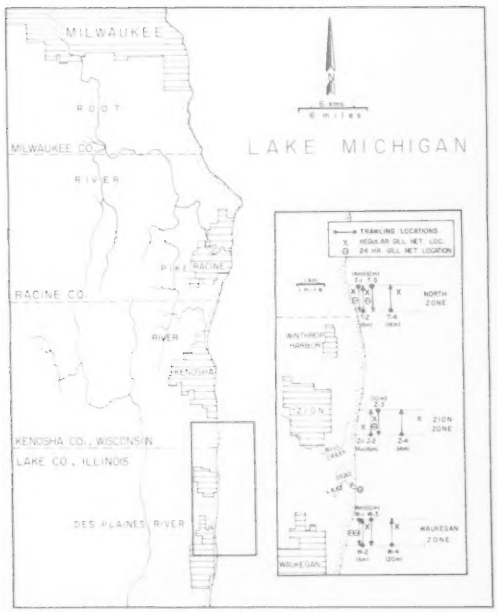


FIG. 1. A map showing sites (T,Z,W) and depths at which fishes examined from Lake Michigan were collected.

*Acanthocephalus jacksoni* Bullock 1962 in Amin (1975a). Fishes were brought to the laboratory on ice and dissected within 24–48 h. Acanthocephalan parasites were systematically recovered from previously mapped host intestines, refrigerated in distilled water overnight, stained in Mayer's acid carmine, cleared in terpiniol, and mounted in Canada balsam.

Throughout the text the mean values refer to the number of parasites recovered per number of fishes examined, and each season is defined as: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November).

## Results and Discussion

### Distribution in Host Species

Of 18 fish species examined in this study, 14 species belonging to eight families were found infected with 6426 *E. salmonis* (1674 males, 4752 females) (Table 1).

New host records are reported for chinook salmon, *Oncorhynchus tshawytscha*, and slimy sculpin, *Cottus cognatus*. First host records for Lake Michigan include alewife, *Alosa pseudoharengus*; bloater, *Coregonus hoyi*; rainbow trout, *Salmo gairdneri*; rainbow smelt, *Osmerus mordax*; and spottail shiner, *Notropis hudsonius*. *Echinorhynchus salmonis* did not occur in the 11 gizzard shad, *Dorosoma cepedianum*; 3 carp, *Cyprinus carpio*; 1 black bullhead, *Ictalurus melas*; and 1 trout perch, *Percopsis omiscomaycus* examined.

*Echinorhynchus salmonis* was the dominant parasite in all infected hosts except white sucker, *Catostomus commersoni*, in which *Acanthocephalus parksidei* Amin, 1974 was more abundant. It infected salmonid fishes considerably more heavily than nonsalmonids. Coho salmon, *Oncorhynchus kisutch*, was particularly heavily infested with up to 796 *E. salmonis* per host. Some nonsalmonids, however, appeared to regularly sustain moderate infections, e.g. *Osmerus mordax*. Parasite maturation was not restricted to salmonids; females with embryos were recovered from all hosts.

### Seasonal Distribution and Development

Complete monthly collections were available for *O. mordax*. In that host, no pronounced seasonal periodicity in the rate and intensity of infection with *E. salmonis* was observed (Fig. 2). However, a slight increase in both variables was observed during early autumn and spring as well as in rate during December. Prevalence in other fish species was highest during winter. Intensity of infection was lowest during the autumn and

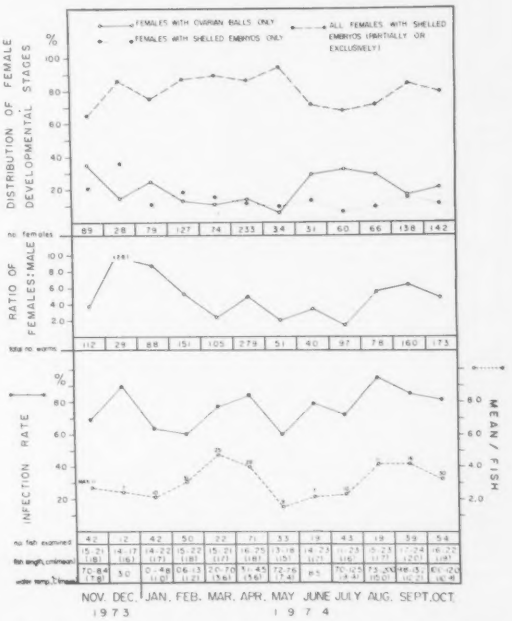
DISTRIBUTION OF FEMALE DEVELOPMENTAL STAGES
FEMALES WITH OVARIAN BALLS ONLY
FEMALES WITH SHELLED EMBRYOS ONLY
ALL FEMALES WITH SHELLED EMBRYOS (PARTIALLY OR EXCLUSIVELY)
RATIO OF FEMALES:MALE
INFECTION RATE
MEAN / FISH
NOV. DEC. JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT.
1973
1974

FIG. 2. Seasonal periodicity of *Echinorhynchus salmonis* in rainbow smelt, *Osmerus mordax*. Seasonal distribution of female developmental stages (top), seasonal sex ratio (middle), and rate and intensity of infection (bottom, maximum number of worms recovered per fish is indicated on lower panel).

TABLE 1. Lake Michigan fishes infected with *Echinorhynchus salmonis*. Fishes were examined between November 1973 and October 1974. No. coll. = number collected; Ovar. = ovarian; Emb. = embryos.

| Host | | | | *Echinorhynchus salmonis* | | | | | % females with | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | No. exam. | Month | Length (*cm*) mean (range) | Infect rate (%) | No. coll. (♂:♀) | Month | Mean/ fish | Max/ fish | Ovar. balls only | Both | Emb. only | *N* |
| Clupeidae | | | | | | | | | | | | |
| *Alosa pseudoharengus* | 149 | XI, V–IX | 16(13–19) | 13 | 36 (1:1.57) | XI, V–VIII | 0.23 | 7 | 86 | 14 | 0 | 22 |
| Salmonidae | | | | | | | | | | | | |
| *Coregonus clupeaformis* | 8 | I, III, IX | 41(27–61) | 100 | 176 (1:1.71) | I, III, IX | 22.00 | 56 | 47 | 48 | 5 | 111 |
| *C. hoyi* | 79 | XI, III, VII–X | 23(8–28) | 96 | 984 (1:3.60) | XI, III, VII–X | 12.46 | 54 | 15 | 79 | 6 | 770 |
| *Oncorhynchus kisutch* | 15 | III, IV, IX | 43(29–50) | 100 | 2104 (1:2.06) | III, IV, IX | 140.27 | 796 | 27 | 51 | 22 | 1416 |
| *O. tshawytscha*[a] | 6 | VIII, IX | 62(53–68) | 100 | 320 (1:2.11) | VII, IX | 53.33 | >73[b] | 2 | 79 | 19 | 217 |
| *Salmo gairdneri* | 8 | XI, I–III VIII | 46(29–59) | 75 | 305 (1:1.70) | XI, I–III | 38.12 | 85 | 28 | 54 | 18 | 192 |
| *S. trutta* | 16 | I–III, V, VIII, X | 40(24–63) | 100 | 263 (1:2.76) | I–III, V, VIII, X | 16.44 | 62 | 26 | 57 | 17 | 193 |
| *Salvelinus namaycush* | 7 | XI, III, V VIII | 65(60–68) | 100 | 573 (1:7.55) | XI, III, V, VIII | 81.86 | 224 | 3 | 92 | 5 | 506 |
| Osmeridae | | | | | | | | | | | | |
| *Osmerus mordax* | 446 | All | 18(11–25) | 75 | 1363 (1:4.20) | All | 3.06 | 31 | 19 | 67 | 14 | 1101 |
| Cyprinidae | | | | | | | | | | | | |
| *Notropis hudsonius* | 15 | XII, I, III | 11(9–13) | 0 | 0 | — | 0 | 0[c] | | | | |
| Catostomidae | | | | | | | | | | | | |
| *Catostomus commersoni* | 24 | I, III–V, IX | 39(24–58) | 42 | 22 (1:2.14) | I, III–V, IX | 0.92 | 4 | 80 | 20 | 0 | 15 |
| Cadidae | | | | | | | | | | | | |
| *Lota lota* | 1 | IX | 40 | 100 | 18 (1:5.00) | IX | 18.00 | 18 | 20 | 47 | 33 | 15 |
| Cottidae | | | | | | | | | | | | |
| *Cottus cognatus*[a] | 37 | III | 9(7–10) | 73 | 253 (1:3.02) | III | 6.84 | 38 | 17 | 51 | 32 | 190 |
| Percidae | | | | | | | | | | | | |
| *Perca flavescens* | 11 | XI | 17(14–24) | 45 | 9 (1:0.80) | XI | 0.82 | 3 | 75 | 25 | 0 | 4 |

[a]New host records.
[b]One chinook salmon examined during the pilot study, April 1973, yielded >223 *E. salmonis*.
[c]Of three spottail shiners examined during the pilot study, two yielded four *E. salmonis*.

highest during winter and spring (Table 2). The pattern expressed in Table 2 represented that of eight of the nine fish species included. Only the bloater was most frequently and heavily infected during the summer (29 examined) with autumn (47 examined) and spring (only 3 examined) ranking second and third, respectively.

No seasonal periodicity in development and maturation was observed in the rainbow smelt indicating that recruitment and maturation continued throughout the year (Fig. 2). The young of the amphipod intermediate hosts, *Pontoporeia* (Van Cleave 1920 and work in progress), were shown by Larkin (1948) to be borne during the winter and to survive for more than 2 yr. Female *E. salmonis* in all developmental stages were recovered from other host species throughout the year, with the highest percentage of females with ovarian balls found during the winter and with embryos (partially or exclusively) during spring and summer (Table 2).

The above information suggests that recruitment and maturation are associated with colder and warmer temperatures, respectively. In *O. mordax*, however, the percentage of females with only ovarian balls increased during the summer (Fig. 2). The observed absence of pronounced periodicity is in agreement with Chubb's (1964) conclusions that the absence of cyclic fluctuations of *E. clavula* in fishes of Llyn Tegid is related to the lack of pronounced seasonal temperature fluctuations. Neither Llyn Tegid nor Lake Michigan freeze over in winter. Marked seasonal periodicity of *E. salmonis* was documented in fishes from bodies of water that freeze over in winter, e.g. Tedla and Fernando (1969, 1970, Lake Ontario) and Seng (1975, Cold Lake, Alberta). Other examples are included in Chubb (1964), James and Srivastava (1967), and Kennedy (1975). The observed tendency of somewhat greater recruitment and maturation during colder and warmer months, respectively, was documented by Awachie (1972) for *E. truttae* and by Kennedy (1972) for *Pomphorhynchus laevis*. Factors other than temperature that affect periodicity (or lack of it) suggested by this study and others in progress include host feeding behavior (as influenced by temperature and age or size) and the seasonal availability of infected intermediate hosts. Other contributing factors suggested by others include the effect of tidal cycles on the seasonality of *E. gadi* through its effect on the amphipod fauna (James and Srivastava 1967) and the effect of the total number of acanthocephalans, number of

cestode parasites (if any), and host size on the maturation cycle of *E. salmonis* (Seng 1975).

The absence of pronounced seasonal periodicity of *E. salmonis* infections appears to be of a dynamic and not a static nature and could probably be explained, at least in part, by Kennedy's (1975) observation (on *P. laevis*) that the increased establishment of worms and decreased host feeding activity during the colder months are balanced by worm die-offs and increased host feeding activity later in the warm period.

### Sex Ratio

In almost all cases, females were proportionally more numerous than males and exceptionally so in lake trout, *Salvelinus namaycush*.

Burlingame and Chandler (1941), Awachie (1966), and Amin (1975b) observed that male *Moniliformis dubius*, *E. truttae*, and *A. parksidei*, respectively, were eliminated earlier than females, resulting in a sex ratio in favor of more females towards the end of the infectious period of the cycle. In the smelt, seasonal sex ratio was nearest to even during spring and summer but somewhat higher during autumn and winter, the "wrong seasons" (Fig. 2). This trend could not be explained except in part by the small sample size during some months, e.g. December. A similar trend was previously observed by Kennedy (1972) for *P. laevis* in goldfish, *Carassius auratus*. The seasonal sex ratio in the nine other fish species was in agreement with the expected pattern (as quoted above) and was nearest to even during winter and gradually shifted in favor of females during spring and summer (Table 2).

### Distribution in Host Intestine

On the average, 50% or more of *E. salmonis* were concentrated in the posterior-most intestinal region (no. 6) of smelt and other host species (Table 3). Distribution in host intestine might have been partially related to host age, among other factors. For example, in older brown trout *Neoechinorhynchus rutili* was proportionally more numerous in the post pyloric cecae than in the pyloric cecae (Thomas 1964a) and the reverse pattern was observed for *E. salmonis* infections in whitefish (Seng 1975).

No pronounced seasonal displacement of worms posteriorly along the intestine of various hosts was observed (Table 3). This appears to be a function of the continuous recruitment, maturation, and elimination of worms throughout the year. The fact that the percent distribution of worms in intestinal regions 4 and 5 was highest during winter and early spring and that it progressively increased in intestinal region 6 during the warmer months (Table 3) suggests a posterior migration with maturation. This is further supported by the observation that mature female *E. salmonis* were usually located more posteriorly than anteriorly. For example, in a January collection from rainbow trout the percent distribution of females with ovarian balls, with mixed ovarian balls and embryos, and with embryos only was 80, 20, and 0 in intestinal regions 4 and 5 combined and 12, 65, and 23 in intestinal region 6.

### Host Size

An overall increase in frequency and intensity of infection with *E. salmonis* with increased host size (age) was observed where collections during the same or comparable periods were available. For example, of 20 smelt examined during July and averaging 12 cm in total length (range 11–14) 50% were infected with 11 worms (mean of 0.55 per fish). In a collection of larger smelt during the same month, 91% of 23 fishes averaging 18 cm in length (15–23) were infected with 86 worms (mean 3.74).

Evidence from this study suggests that the observed pattern of heavier parasitic infection with increased host size (age) is of two major types. The first type involves a relatively stable host feeding behavior by age where the heavier parasitic load in larger fish becomes simply a function of the greater volume of food ingested including the infected intermediate host. A study by Hart (1931) on lake whitefish, *Coregonus clupeaformis*, in Ontario gives an example of this type. The amphipod content (including *Pontoporeia hoyi*) in the diet of the whitefish infected with *Echinorhynchus* sp. notably increased with fish size (13–55 cm long fishes in five size-classes). Using eight age-classes of whitefish (2–9 yr) from Cold Lake, Seng (1975) showed that a similar increase in the prevalence of *P. affinis* in the diet of older fish corresponded with heavier infections with *E. salmonis*.

The second type involves a basic change in host feeding behavior by age. The larger piscivorous salmonids, e.g. salmon and trout, represent this type. These fishes, particularly the larger ones, were heavily infected with *E. salmonis* and an alternative route of infection other than through the basic cycle involving the amphipod intermediate host is probably involved. This would be through the transfer of adult *E. salmonis* from one species to another through predation. Younger salmonids ingest significant proportions of invertebrates (including amphipods) which are virtually absent in the diet of larger fish. Such a feeding pattern was demonstrated in the coho salmon and/or lake trout in Lake Michigan by

TABLE 2. Seasonal data of *Echinorhynchus salmonis* from Lake Michigan fish species excluding smelt.

| | | Winter | Spring | Summer | Autumn |
|---|---|---|---|---|---|
| Rate and intensity of infection | Avg | 14(93), 317(22.64)[a] | 127(60), 3148(24.79) | 92(46), 941(10.23) | 128(51), 657(5.13) |
| Sex ratio | Avg | 1:1.66(317)[b] | 1:2.35(3148) | 1:3.59(941) | 1:3.53(657) |
| | Range[c] | 1:1.48(166) in rainbow trout<br>1:2.03(110) in whitefish | 1:1.50(65) in whitefish<br>1:6.63(374) in lake trout | 1:1.92(266) in chinook salmon<br>1:10.87(190) in lake trout | 1:3.34(504) in bloater<br>1:9.00(40) in coho salmon |
| Developmental stages | Avg | 48, 39, 13(198)[d] | 23, 57, 20(2207) | 11, 83, 6(736) | 12, 76, 12(512) |
| | Range[c] | 42, 41, 17(99) in rainbow trout<br>54, 33, 13(24) in brown trout | 3, 91, 6(325) in lake trout<br>41, 59, 0(39) in whitefish | 1, 96, 3(174) in lake trout<br>18, 79, 3(381) in bloater | 5, 64, 31(42) in chinook salmon<br>12, 79, 9(388) in bloater |

[a]Number of fish examined (percent infected), number of acanthocephalans recovered (mean per fish).
[b]Male:females ($N$ = number of males and females).
[c]Only the larger sample sizes from hosts represented in more than one season (all salmonids, alewife, and white sucker) are included.
[d]Percent females with ovarian balls only, with ovarian balls and shelled embryos, with shelled embryos only ($N$ = number of females).

TABLE 3. Seasonal distribution (percent) of *E. salmonis* ($N$ = number of males and females) in the intestine[a] of some Lake Michigan fishes.

| | Rainbow smelt | | | | | Other fish species[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | $N$ | 3 | 4 | 5 | 6 | $N$ |
| Winter | 0 | 2 | 26 | 72 | 268 | 0 | 28 | 35 | 37 | 312 |
| Spring | 0 | 1 | 9 | 90 | 435 | 2 | 18 | 38 | 42 | 3131 |
| Summer | 0 | 0 | 25 | 75 | 216 | 0 | 2 | 34 | 64 | 933 |
| Autumn | 0 | 4 | 15 | 81 | 445 | 1 | 3 | 15 | 81 | 598 |
| Avg | 0 | 2 | 17 | 81 | 1364 | 2 | 14 | 34 | 50 | 4974 |

[a]Intestine of all tabulated species was divided into six regions except the sculpin (with four). Regions 1–3 divide the first two intestinal limbs into three equal parts and regions 4–6 divide the long posteriormost limb leading to the vent into three equal parts.
[b]Not including data from white sucker, burbot, yellow perch, spottail shiner (small sample size).

Peck (1974), in Lake Superior by Dryer et al. (1965), in Ontario by Martin (1970), and in Wisconsin by McKnight and Serns (1974). The use of transport hosts as such was previously suggested by Chubb (1964) for *E. clavula* and established for other acanthocephalans of fish by Petrochenko (1956). The transfer of *E. salmonis* was experimentally demonstrated between coho salmon and brook trout (*S. fontinalis*) from Lake Michigan by Hnath (1969). Older Lake Michigan coho salmon and/or lake trout feed almost exclusively on a fish diet composed chiefly of smelt, alewife, and stickleback (species not given) (Harney and Norden 1972) as well as *Cottus* spp. (including *C. cognatus*) and *Coregonus* spp. (Van Oosten and Deason 1938). These prey species, with the exception of sticklebacks which were not sampled, were all infected with *E. salmonis* and probably represent important transfer hosts. Seng (1975) estimated that about one eighth of *E. salmonis* flow in the Cold Lake ecosystem was through transport hosts, primarily sticklebacks.

## NONINTESTINAL INFECTIONS

A total of 40 worms were recovered from the body cavity of smelt. Most were identified as *E. salmonis* and one male and one female appeared to be *A. parksidei*. A number of younger stages were also recovered. Infection in this site was more frequent during autumn and spring than during other seasons. Most specimens were females (38), half of which were gravid. A small number of worms appeared in a degenerate condition and almost all were enclosed within envelopes of undetermined origin.

TABLE 4. Seasonal infections of male and female rainbow smelt, *Osmerus mordax*, with *E. salmonis*.

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | No. exam. | % infect. | Worms recover. | Mean/ fish | No. exam. | % infect. | Worms recover. | Mean/ host |
| Winter | 26 | 65 | 52 | 2.00 | 78 | 65 | 218 | 2.79 |
| Spring | 33 | 76 | 78 | 2.36 | 93 | 77 | 359 | 3.86 |
| Summer | 56 | 82 | 151 | 2.71 | 25 | 72 | 62 | 2.48 |
| Autumn | 67 | 84 | 235 | 3.51 | 68 | 73 | 208 | 3.05 |
| Total | 182 | 79 | 516 | 2.84 | 264 | 72 | 847 | 3.21 |

TABLE 5. The relationship between the sex of bloater, *Coregonus hoyi*, coho salmon, *Oncorhynchus kisutch*, and slimy sculpin, *Cottus cognatus*, and infections with *E. salmonis*.

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | No. exam. | % infect. | Worms recover. | Mean/ fish | No. exam. | % infect. | Worms recover. | Mean/ host |
| Bloater | 35 | 97 | 347 | 9.91 | 44 | 95 | 637 | 14.48 |
| Coho salmon | 7 | 100 | 762 | 108.86 | 8 | 100 | 1342 | 167.75 |
| Sculpin | 25 | 72 | 135 | 5.40 | 12 | 75 | 118 | 9.83 |

### EFFECT OF HOST SEX

Rate of infection was similar in both sexes but intensity of infection was somewhat higher in female than male smelt, particularly during the spring breeding season (Table 4) as well as in bloaters, coho salmon, and sculpins (Table 5). Most of these hosts were sexually mature and the trend of heavier infections in females, at least during the mating season as in smelt, might be related to the observations by Thomas (1964b) for *N. rutili* in the brown trout. He observed that 3-yr-old or older trout that were spawning or recovering from spawning were more heavily infected with worms (although they consumed significantly less food then) than males as well as females during other times of the year. This was attributed to the lower physiological resistance of the female as mediated through decreased estrogen level during breeding rather than to ecological or behavioral differences (Thomas 1964b).

### PATHOGENICITY

In the present investigation slight inflammation of host intestinal wall was occasionally observed at the site of *E. salmonis* attachment. No further observations were made. Infection with *E. salmonis* under conditions prevailing in the study area was not a significant factor affecting the health or survival of its fish hosts.

*Echinorhynchus salmonis* appears, however, to cause serious damage, at least occasionally, to its coregonid and salmonid hosts, particularly when present in great numbers. Localized lesions at attachment sites involving hyperemia and leukocytosis were reported by Petrochenko (1956) and Bauer (1961); perforation occasionally occurred. Host weight loss, retarded growth, and malnutrition were reported by Petrushevski and Shulman (1961), Bauer (1953 in Petrochenko, 1956), and Petrushevski and Kogteva (1954 in Collins and Dechtiar, 1974), respectively.

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# Daily Rings in Otoliths of Three Species of *Lepomis* and *Tilapia mossambica*[1]

BRUCE D. TAUBERT[2] AND DANIEL W. COBLE

*Wisconsin Cooperative Fishery Research Unit,[3] College of Natural Resources, University of Wisconsin, Stevens Point, Wis. 54481, USA*

TAUBERT, B. D., AND D. W. COBLE. 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Board Can. 34: 332–340.

Daily rings formed on otoliths of known-age, laboratory-raised pumpkinseed (*Lepomis gibbosus*), green sunfish (*L. cyanellus*), bluegill (*L. macrochirus*), and mozambique mouthbrooder (*Tilapia mossambica*) for at least 176, 170, 125, and 60 days, respectively. Subdaily rings found in young laboratory and wild fish were easily distinguished from daily rings. Width of daily rings on otoliths of green sunfish was linearly related to daily increase in length of fish, but the number of rings was a product of age of fish only, not length of fish or otolith radius. Growth and daily ring formation on otoliths in wild bluegill and largemouth bass (*Micropterus salmoides*) appeared to be similar to those in laboratory-raised fish. Otoliths of green sunfish held under simulated winter conditions ceased to produce daily rings, but did form an annulus. Two kinds of otolith tissue were present in most of the larger laboratory fish and wild bluegill but were not observed in wild largemouth bass. The first type, present in all areas of the otolith except the extreme posterior end, was translucent and had well-defined daily rings. The second type, present only in the posterior end, was opaque and had poorly etched daily rings that were difficult to discern. Both tissues were calcium carbonate in the aragonite form. Daily rings were found on otoliths of fish held at constant temperature. Results of experiments with young mouthbrooders held under various light–dark and feeding cycles suggested that a 24-h light–dark cycle that entrained an internal, diurnal clock was required for daily ring production.

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Des anneaux se sont formés quotidiennement sur les otolithes de crapets-soleils (*Lepomis gibbosus*), de crapets verts (*L. cyanellus*), de crapets arlequins (*L. macrochirus*) et de tilapias de Mozambique (*Tilapia mossambica*) d'âge connu et élevés en laboratoire, pendant au moins 176, 170, 125 et 60 jours respectivement. On pouvait facilement distinguer des anneaux quotidiens les anneaux subquotidiens rencontrés chez les jeunes poissons de laboratoire ou sauvages. La largeur des anneaux quotidiens sur les otolithes des crapets verts est en relation linéaire avec l'augmentation journalière de la longueur des poissons, mais le nombre d'anneaux est le produit de l'âge seulement du poisson, non de sa longueur ni du rayon de l'otolithe. La croissance et la formation quotidienne d'anneaux sur les otolithes de crapets arlequins et d'achigans à grande bouche (*Micropterus salmoides*) semblent identiques à celles des poissons élevés en laboratoire. Les otolithes de crapets verts maintenus dans des conditions simulées d'hiver cessent de produire des anneaux quotidiens, mais forment un annulus. On observe deux sortes de tissus otolithaires chez la plupart des grands poissons de laboratoire et chez les crapets arlequins sauvages, mais non chez l'achigan à grande bouche sauvage. Le premier type, présent dans toutes les régions de l'otolithe sauf l'extrémité postérieure, est translucide et possède des anneaux quotidiens bien définis. Le second type, présent seulement dans l'extrémité postérieure, est opaque et possède des anneaux quotidiens mal définis et difficiles à reconnaître. Les deux tissus sont constitués de carbonate de calcium dans sa forme aragonite. On trouve des anneaux quotidiens sur les otolithes de poissons maintenus à température constante. Les résultats d'expériences avec de jeunes tilapias soumis à divers cycles jour–nuit et d'alimentation suggèrent qu'un cycle jour–nuit de 24 h déclenchant une horloge nycthémérale interne est nécessaire à la production d'anneaux quotidiens.

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[2]Present address: Massachusetts Cooperative Fishery Research Unit, College of Food and Natural Resources, University of Massachusetts, Amherst, Mass. 01003, USA.

[3]U.S. Fish and Wildlife Service, Wisconsin Department of Natural Resources, and University of Wisconsin (Stevens Point) cooperating.

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PURPOSES of this study were to determine if daily rings were produced in otoliths of known-age, laboratory-raised pumpkinseed (*Lepomis gibbosus*), green sunfish (*L. cyanellus*), bluegill (*L. macrochirus*), and mozambique mouthbrooder (*Tilapia mossambica*), and if so, to determine if daily rings can be used to back-calculate subseasonal age and growth of fish; to determine if daily rings were formed in winter; to compare daily rings and otolith growth of laboratory and wild fish, and to investigate the factor(s) that may control daily ring production.

Pannella (1971, 1974) reported the presence of daily rings in otoliths of some marine and tropical fish and believed that they were a universal property of fish otoliths. Brothers et al. (1976) found daily rings in otoliths of known-age northern anchovy (*Engraulis mordax*) and California grunion (*Leuresthes tenuis*) and suggested their presence in several species of fish of unknown age. Struhsaker and Uchiyama (1976) used daily rings in known-age fish to develop a daily growth curve for wild nehu (*Stolephorus purpureus*) up to 160 days old. Daily rings have not been reported for freshwater fish from a temperate climate, and the factors causing daily ring formation have not been determined.

We defined a daily ring in an otolith as a pair of adjacent bands, one light and one dark (Fig. 1). The appearance of light and dark bands is probably caused by daily fluctuations in the ratio of calcium carbonate to otolin (Degens et al. 1969).

## Materials and Methods

### OTOLITH PREPARATION

Of the three pairs of otoliths in the inner ear of fish, the sagittae, in the sacculus, the largest otoliths and only the ones suitable for age studies, were used. A variable (10–70×) binocular dissecting microscope was used to aid in extracting otoliths from fish 4–25 mm in total length (TL). The fish's head was removed with forceps and spread on the microscope stage, where, under transmitted light, the otoliths appeared as two shiny, oval objects. We removed the otoliths from larger fish through a transverse incision extending half way through the fish's head, along the posterior edge of the operculum.

Two preparation techniques were used, depending on the size of the otolith. Small otoliths, which were fragile and difficult to handle, were ground into thin sagittal sections to expose their lateral surface (Fig. 2). They were secured to a glass microscope slide with Canada balsam, with the proximal surface against the slide. The slide served as a holder while the otoliths were ground by hand against a mixture of no. 600 carborundum powder and water on a glass surface. Next, the otoliths were polished on a rotating wheel against no. 4000 aluminum oxide on a wet felt pad. By alternately grinding, polishing, and observing the otoliths through a microscope, we were able to determine when the nucleus (the portion of the otolith produced during the embryonic period) was exposed. When the nucleus was reached, the preparation (otolith plus slide) was immersed in 1% HCl for 15–45 s. The acid etching removed some of the calcium carbonate and made the rings more distinct. The entire procedure for each pair of otoliths required about 5 min.

Larger otoliths were ground perpendicular to the lateral surface and parallel to the axis from the posterior to anterior edges (frontal section). The dorsal edge of the otolith was ground as described above, but the grinding was stopped just before the nucleus was reached. Then the Canada balsam was heated, and the otolith was turned and the newly ground surface was secured to the slide. Grinding was resumed on the exposed side of the section until the nucleus was reached. The preparation was immersed, as before, in 1% HCl for 15–45 s. In some large otoliths that were poorly etched, we were able to make rings more distinct by placing a drop of glycerine on the otolith and using a cover slip, or by immersing the preparation in water or ethanol for a few hours. All otoliths were viewed under a microscope at a magnification of 400× or greater, and the mean of three ring counts was recorded.

### AGE DETERMINATION FROM RING NUMBERS

Pumpkinseed and bluegill eggs and larvae were collected from nests in Wisconsin lakes, and green sunfish and mouthbrooders were spawned in the laboratory. Fish were held in tanks of water of 24–27°C with a photoperiod of 15 h light and 9 h dark (L15:D9). Based on information from Morgan (1951) and Balon (1959), ages of bluegill and pumpkinseed at swim-up or the beginning of the juvenile period (Balon 1975) were estimated to be 6 days. Age of green sunfish at swim-up was known to be 6 days. Mouthbrooders left the female's mouth 10–12 days after fertilization of the eggs. Juvenile *Lepomis* were fed plankton and freshly hatched or frozen brine shrimp (*Artemia salina*), and mouthbrooders were fed dry trout food.

Fish were killed daily for 2 wk after swim-up, and thereafter at about 2-wk intervals, and stored in 70% ethanol or 70% ethanol plus 10% glycerine. Otoliths from these fish were sectioned both sagittally and frontally.

### DAILY RINGS RELATED TO AGE AND SIZE OF FISH

Four groups of 15 green sunfish were killed 31, 45, 59, and 89 days after swim-up. All fish were from the same spawning, and a wide range of lengths was selected within each group. Fish were measured to the nearest 0.1 mm TL under a dissecting microscope. A frontal section of both otoliths was prepared, otolith radius (distance from nucleus to posterior edge) was measured to the nearest 0.01 mm, and the rings were counted.

Six otoliths from the 60 fish were discarded because they were broken or the grinding had failed to

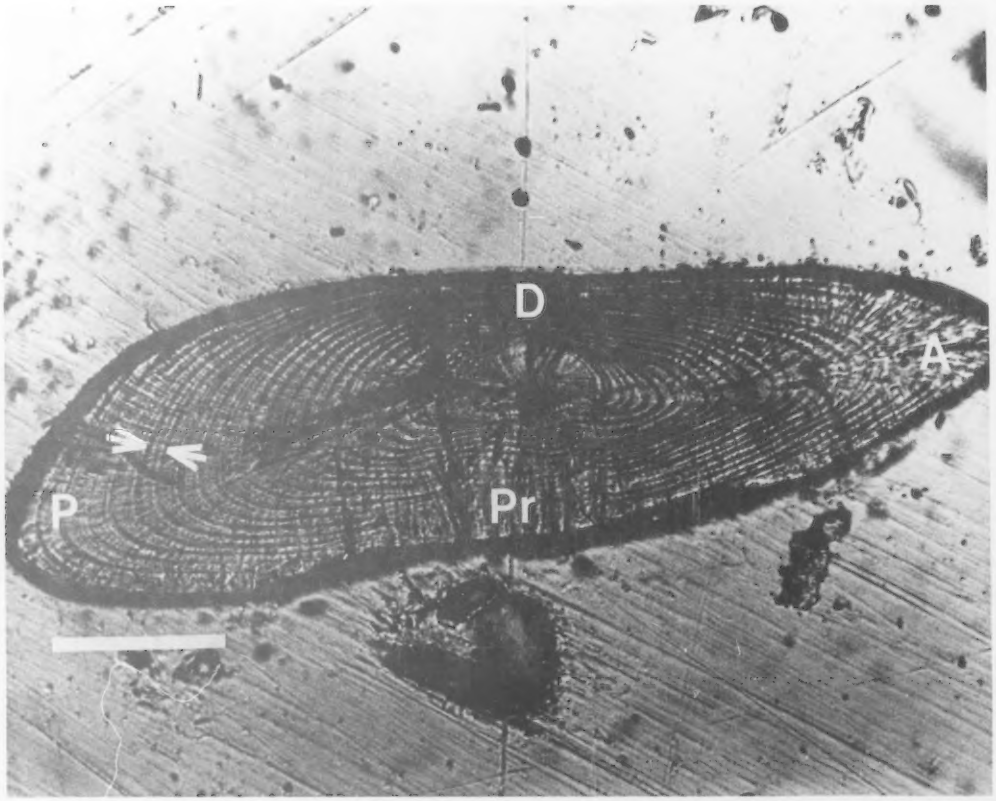


FIG. 1. Frontal section of otolith from 24-day-old pumpkinseed (*Lepomis gibbosus*). Posterior end, P; proximal, Pr; distal, D. Compression of daily rings can be seen in the distal and proximal areas. Subdaily rings can be seen between the prominent daily rings (arrows). Bar scale = 0.1 mm.

reveal the nucleus. In the group sacrificed 89 days after swim-up, seven otoliths did not reveal a continuum of rings due to the presence of opaque tissue (discussed below) and were also discarded.

### Effects of Simulated Winter on Daily Ring Production

To determine if daily rings were produced in otoliths of wintering fish, we exposed 90 green sunfish to simulated winter conditions in an environmental chamber. Water temperature was reduced 1°C/day, from 25 to 4°C, and the photoperiod from L15:D9 to L10:D14. Fish were fed frozen brine shrimp daily, in excess, until water temperature reached 4°C. At 4°C the fish were nearly inactive and ate little; therefore, feedings were reduced to two per week. A control group was held in the laboratory at 25°C, L15:D9, and fed frozen brine shrimp daily, in excess. Both groups were from the same spawning.

After the experimental group had been held at 4°C for 60 days, they were reacclimated to control conditions. Two fish from the experimental group were killed semiweekly, and fish from the control group less frequently.

### Otolith Growth and Ring Formation in Wild Fish

We prepared sagittal sections of otoliths from largemouth bass (*Micropterus salmoides*) from the Lake Mills (Wisconsin) National Fish Hatchery. We estimated the largemouth bass to be 60 days old on the basis of the date of stocking of adults, records of water temperature, and observations by hatchery attendants of nesting activity.

Bluegill were collected from two Wisconsin lakes — Lake Thomas, Portage County, and Little Musky Lake, Oneida County. Total lengths of the fish and their otoliths were measured to the nearest 1.0 and 0.01 mm, respectively. Otoliths from largemouth bass from Sunset Lake, Portage County, Wisconsin, were also examined.

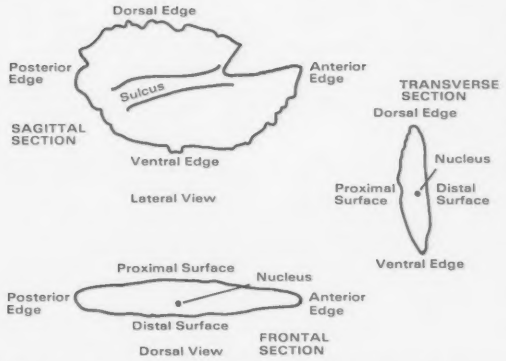


FIG. 2. Diagrammatic sections of an otolith of genus *Lepomis*.

### ENVIRONMENTAL FACTORS INFLUENCING DAILY RING FORMATION

Effects of different light–dark cycles, constant light, a feeding cycle, and an internal, diurnal clock on daily ring formation in mouthbrooders were tested for 60 days. Adult mouthbrooders were put in a 70-$\ell$ aquarium at L15:D9 and a water temperature of 26°C. After they spawned and the females had mouthed the eggs, the females were held under constant light and 26°C. Young from three spawnings were used in the experiments.

When the young fish left a female's mouth, groups of about 50 were put into each of six tanks under different conditions. The tanks contained standing water that was aerated and circulated through a box filter containing activated charcoal and polyester floss. Tanks were cleaned, and automatic feeders were filled with dry trout food at different times every 2 days. Otoliths from weekly samples were sectioned frontally and sagittally.

Group 1 was held under constant light and constant water temperature of 27°C, and was fed at 3-h intervals. Since no 24-h stimulus was present, we believed that any daily ring formation would be the product of an internal, diurnal clock.

Group 2 was held under the same conditions, except that the fish were fed at 3-h intervals, with a 9-h break in feeding every 24 h. The fish were held in a tank within a tank (the inner tank had a screen bottom with mesh size larger than the food particles, but smaller than the fish) so that food would not be available during the break in feeding. We expected to find one ring every 24 h if the stimulus causing daily ring formation were a daily change in food availability.

Group 3 was held under L24:D12, 27°C, and fed automatically every 6 h. If a light–dark cycle were responsible for daily ring formation, we expected to find a ring formed every 36 h.

Group 4 was held at L15:D9, 27°C, and fed automatically every 6 h. The purpose was to determine if isolation in an environmental chamber would alter daily ring formation, which had previously been observed in fish held in the laboratory under about the same conditions of light and temperature.

Group 5 was held and fed in the same manner as group 4 except that the fish were in the laboratory and experienced daily fluctuations in laboratory activity.

Group 6 was held in the laboratory under L15:D9, 25–26°C. It differed from group 5 only in that the fish were fed at irregular times during the day.

Temperature was not used as a cyclic stimulus because fish used in other experiments had produced daily rings without daily fluctuations in temperature.

## Results and Discussion

### AGE DETERMINATION FROM RING NUMBERS

Daily rings were produced in otoliths of pumpkinseed, green sunfish, bluegill, and mouthbrooders for at least 176, 170, 125, and 60 days, respectively. In *Lepomis*, the first daily ring was produced the day of swim-up, and in mouthbrooders it formed approximately when the young left the female's mouth. Brothers et al. (1976) found that the first daily ring in otoliths of *E. mordax* was formed after the yolk sac was absorbed and in *Leuresthes tenuis* after hatch — hence presumably after swim-up in *E. mordax*, but not necessarily in *L. tenuis*.

Small otoliths were disk shaped, and sagittal sections showed daily rings concentric to the nucleus. As the otoliths grew, they became elongated and curved; the distal surface became concave and the proximal surface convex. In frontal sections daily rings were compressed distal and proximal to the nucleus, and individual rings were more prominent in the axis from nucleus to posterior edge (Fig. 1). Otoliths from fish older than about 60 days showed daily rings only in the posterior end.

Subdaily rings were present in all otoliths from fish younger than about 80 days and were more easily seen in frontal (Fig. 1) than in sagittal sections. Weak rings were also present in the nucleus, but their periodicity was not determined. Subdaily rings and those in the nucleus were easily distinguished from daily rings because they were not well defined.

Intersecting daily rings were present in larger sagittae (Fig. 3), consequently some rings found in one area of a sagitta were absent in others. When intersecting rings were found, we examined several areas of an otolith and counted the maximum number of rings. Since intersecting rings were present in both sagittal and frontal sections, they did not seem to be a product of the grinding process.

Two kinds of tissue were present in the otoliths of most of the larger laboratory fish (Fig. 4). The

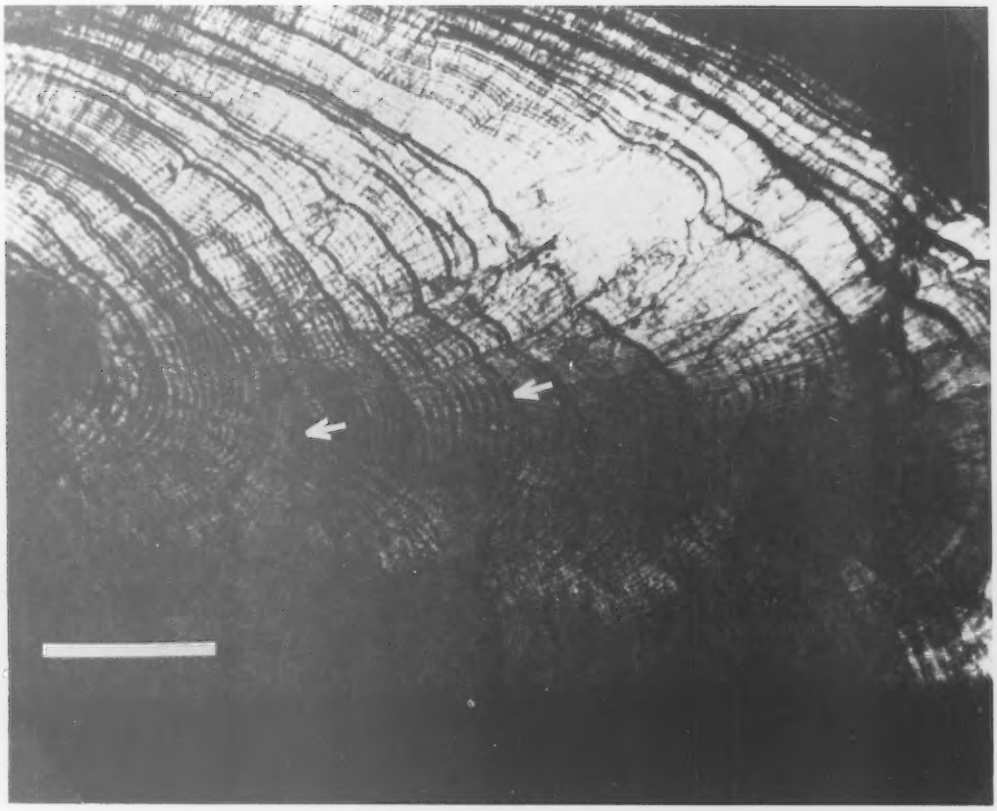

FIG. 3. Frontal section from 176-day-old pumpkinseed with intersecting daily rings (arrows). Bar scale = 0.1 mm.

first, which was present in all areas of the otoliths except the posterior end, was translucent and had well-defined rings. The second, which was present only in the posterior end, was opaque and had rings that were poorly etched and difficult to discern. To determine if the two tissues differed in composition, we broke otoliths from 50 bluegill, separated the two tissues, and sent them to S. W. Baily, Geology Department, University of Wisconsin, Madison, for analysis by X-ray diffraction. He reported that both tissues were calcium carbonate in the aragonite form and could identify no difference between them. Degens et al. (1969) and Irie (1960) also found otoliths to be composed of aragonite crystals. We were unable to determine the duration of daily ring production in pumpkinseed and green sunfish because accurate ring counts could not be obtained from otoliths containing both tissues. No attempt was made to determine the duration of daily ring production in bluegill and mouthbrooders.

## DAILY RINGS RELATED TO AGE AND SIZE OF FISH

The mean number of rings for each group of green sunfish was equal to the number of days from swim-up to death, and within each group the standard deviation of ring number was small (Fig. 5). The number of rings within each group did not vary with length of fish. For example, a 24.1-mm fish in the group killed 59 days after swim-up had the same number of rings (59) as a 43.7-mm fish in the same group. Therefore the number of rings was a function of age in days and not length of fish.

The least squares relation of otolith radius to total length of fish was linear (Fig. 5). Analysis of covariance of least squares regression lines for

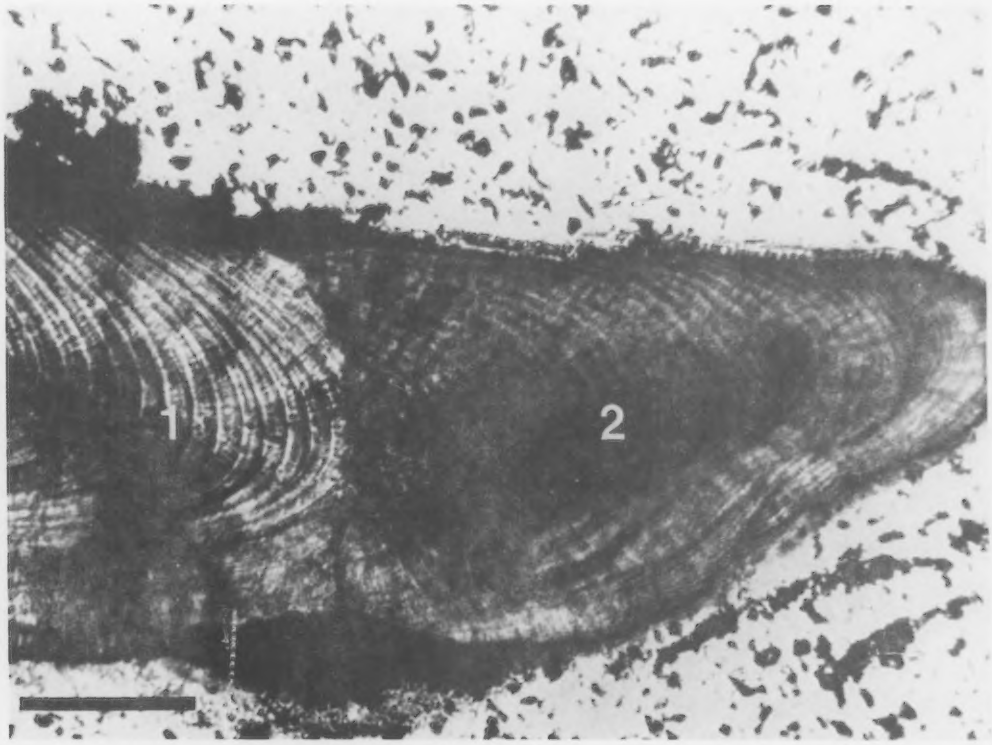


FIG. 4. Frontal section from 81-day-old pumpkinseed. Daily rings are well defined in the tissue near No. 1, and poorly defined in the tissue near No. 2. Bar scale = 0.1 mm.

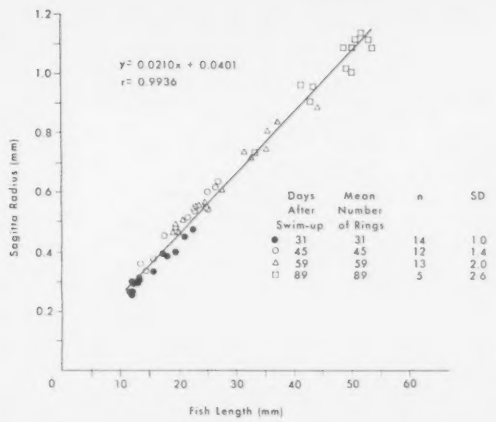


FIG. 5. Otolith radius–body length relation for green sunfish (*L. cyanellus*). Least squares regression, correlation coefficient, and numbers of daily rings are shown in inserts. The functional regression (Ricker 1973) was $y = 0.0211X + 0.0396$.

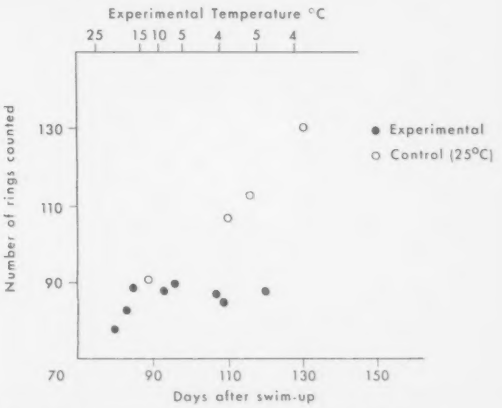


FIG. 6. Number of rings in relation to time and temperature for green sunfish in a simulated winter experiment. Daily ring production ceased after the temperature fell below about 10°C (●), but continued throughout the experiment for control fish held at 25°C (○).

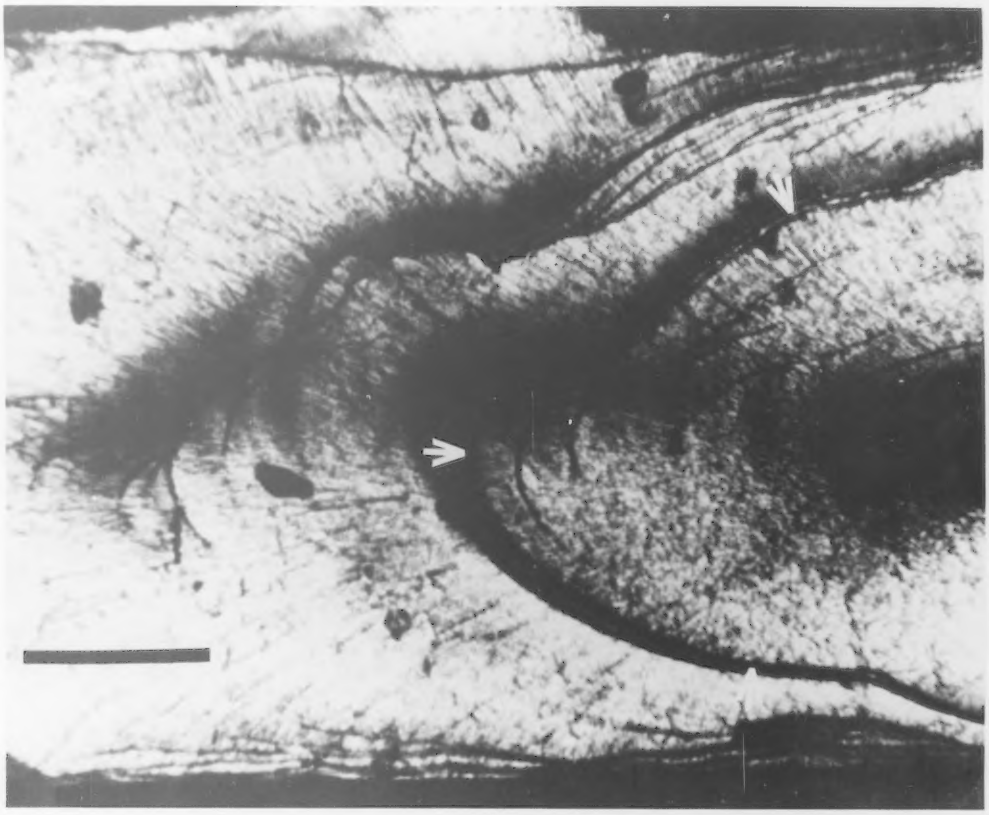

FIG. 7. Laboratory-induced annulus (arrows) in green sunfish otolith. Bar scale = 0.1 mm.

each of the four groups indicated that the relation was the same for all groups ($F_{3,\ 46} = 3.3694$). Since otolith size was related to length of fish, and since number of rings did not vary with length of fish, the number of rings was not a function of otolith size.

### EFFECTS OF SIMULATED WINTER ON DAILY RING PRODUCTION

When water temperature had decreased to about 10°C, daily rings no longer formed in otoliths of experimental green sunfish (Fig. 6). Control fish continued to produce daily rings throughout the experiment. Also, as temperature decreased toward 10°C, daily rings became narrower, indicating that daily growth slowed. Widths of daily rings in otoliths of control fish remained nearly constant.

McComish (1971) found that bluegill did not grow at water temperatures below 10°C, and we showed earlier that the width of a daily ring was a product of daily growth of fish; therefore, we believe that cessation of daily ring formation was a result of cessation of fish growth.

An annulus was produced on scales and otoliths when the experimental fish were returned to 25°C. No such mark was present in otoliths or scales of control fish. The scale annulus was typical for *Lepomis*; circuli became closely spaced as growth slowed, and wider as growth resumed; cutting over of circuli was evident (Regier 1962; Wallin 1957). The annulus in the otolith was a deeply etched band, following the contour of otolith shape (Fig. 7).

### OTOLITH GROWTH AND RING FORMATION IN WILD FISH

Otoliths from the Lake Mills largemouth bass had 55–63 rings. They appeared to be daily rings, not only because their numbers were similar to

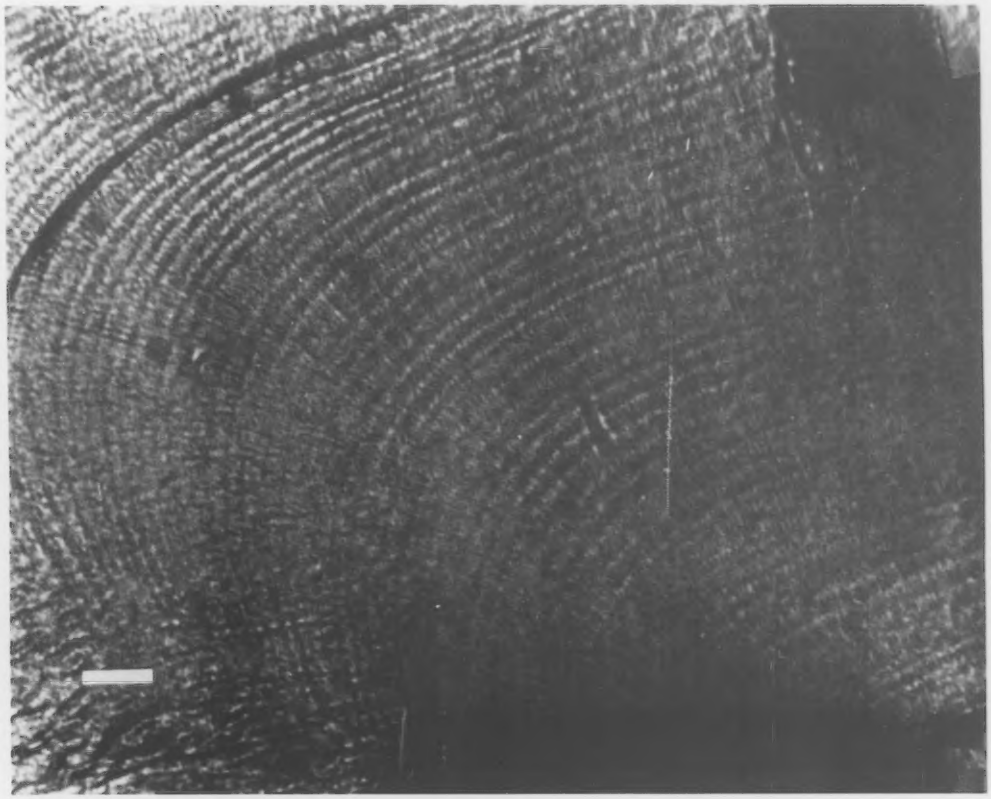

FIG. 8. Rings representative of *T. mossambica* experimental groups 1–3 (see text for description). Bar scale = 0.01 mm.

the estimated age of the fish, but also because their structure was similar to that of the daily rings in known-age laboratory-reared pumpkinseed, green sunfish, and bluegill. Subdaily rings also were found within the first few daily rings of the largemouth bass.

The relation between total lengths of otolith and of fish was different for bluegill from Little Musky Lake and Lake Thomas. The least squares relation of otolith total length ($x$) to fish total length ($y$) was linear for both lakes, but analysis of covariance indicated that the equations were different ($F_{1,\ 145} = 130.4707$). The relation of otolith radius to fish total length in green sunfish also was linear, suggesting that otolith growth in laboratory fish was like that in wild fish. Other studies have shown the relation of otolith growth to fish growth to be linear and population-specific (Rybock et al. 1975; McKern et al. 1974; Messieh 1972; Scott 1973).

Annuli in otoliths of largemouth bass from Sunset Lake were similar to those in otoliths of experimental green sunfish (Fig. 7). Daily rings became narrower as they approached the annulus, indicating a slowing of growth. The description and verification of annuli in scales of centrarchids have been the subject of many studies, but otoliths in this family have received little attention. Annuli in otoliths of green sunfish and largemouth bass may be formed concurrently with those in scales, and they may be used to determine age in years.

The two kinds of tissue described earlier were found in otoliths of the wild bluegill but not in those of wild largemouth bass.

## ENVIRONMENTAL FACTORS INFLUENCING DAILY RING FORMATION

Daily rings were formed in otoliths from fish

in groups 4, 5, and 6, which experienced L15:D9. Otoliths from groups 4 and 5 had subdaily rings, but daily rings were prominent and distinguishable from subdaily rings. Otoliths from group 6 showed well-defined daily rings but subdaily rings were weak or lacking. Again, subdaily rings were more prominent in sagittal sections than in frontal sections.

Daily rings were not formed in groups 1 and 2, and a 36-h ring was not formed in group 3. Otoliths from these groups had many, closely spaced rings (Fig. 8), the rings were not continuous from nucleus to periphery, and they were not visible in all areas of the otolith. Therefore, total numbers of rings could not be determined for these groups. However, in groups 1, 2, and 3 there were at least twice as many (up to 4 times as many) rings as the fish's age in days, even though only a small portion of the otolith showed rings.

The occurrence of daily rings in otoliths of fish exposed to a 24-h light dark cycle and not for fish exposed to constant light or a 36-h cycle, suggests that an internal, diurnal clock was operating, and also, that it had to be entrained by a 24-h light–dark cycle. Groups 3 and 4 differed in the period of the light–dark cycle, 36 and 24 h, respectively. It is difficult to understand why fish in group 3 could sense that the period of the cycle was not 24 h, in the absence of an internal, diurnal clock. These results are consistent with the hypothesis that longer than normal photoperiods have a damping effect on the clock (Saunders 1976); that is, constant light and L24:D12 photoperiods exceeded a light threshhold above which the clock was inoperable.

Apparently an internal, diurnal clock or a cyclic stimulus acting alone was not responsible for formation of rings in the period of the stimulus. If only an internal clock were important, daily rings should have been formed in all groups. If only cyclic feeding were important, daily rings should have been formed in group 2, and should not have been formed in groups 4 and 5. If only the light–dark cycle were important, rings should have been formed in group 3 at 36-h intervals.

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# Temperature and Photoperiod Regulation of Rheotropic Behavior in Prespawning Common Shiners, *Notropis cornutus*

JULIAN J. DODSON

*Department of Biology, University of Waterloo, Waterloo, Ont. N2L 3G1*

AND J. CLIFTON YOUNG

*Department of Statistics, University of Waterloo, Waterloo, Ont. N2L 3G1*

DODSON, J. J., AND J. C. YOUNG. 1977. Temperature and photoperiod regulation of rheotropic behavior in prespawning common shiners, *Notropis cornutus*. J. Fish. Res. Board Can. 34: 341–346.

The upstream orientation and frequency of upstream movement of prespawning common shiners, *Notropis cornutus*, in response to a water current simulated by moving a striped background past the fish were observed at three temperatures (10, 15, 20°C) and two photoperiods (12L:12D and 16L:8D). The greatest frequency of upstream movement was induced by a combination of spring day lengths and warm water temperatures. Further increases in the frequency of upstream movement followed immediate 5°C increases in water temperature. Neither temperature nor photoperiod had a major effect on the frequency of upstream orientation. The significance of environmental regulation of rheotropism as a mechanism of upstream migration is discussed.

DODSON, J. J., AND J. C. YOUNG. 1977. Temperature and photoperiod regulation of rheotropic behavior in prespawning common shiners, *Notropis cornutus*. J. Fish. Res. Board Can. 34: 341–346.

Nous avons observé, à trois températures (10, 15, 20°C) et à deux photopériodes (12J:12N et 16J:8N), l'orientation en amont et la fréquence des mouvements vers l'amont de ménés à nageoires rouges, *Notropis cornutus*, en réponse à un courant d'eau simulé par le déplacement d'un fond de scène rayé près du poisson. La fréquence maximale de mouvements vers l'amont est provoquée par une combinaison de photopériodes printannières et de chaudes températures de l'eau. La fréquence des mouvements vers l'amont augmente encore après augmentations immédiates de 5°C de la température de l'eau. Ni la température ni la photopériode n'ont d'effet majeur sur la fréquence de l'orientation en amont. Nous discutons de la signification du réglage du rhéotropisme dans le milieu comme mécanisme de migration vers l'amont.

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THE term rheotropism is used as a portmanteau word to cover all the reactions that a fish might make in response to a current of water, either directly as a response to water flowing over the body surface or indirectly as a response to the visual, tactile, or inertial stimuli resulting from the displacement of the fish in space (Harden Jones 1968; Arnold 1974). The rheotropic response is composed of an orientational and a kinetic component; fish generally turn to head into a current and adjust their swimming speeds in response to the rate of the current.

Several environmental factors have been observed to affect the rheotropic response, either inducing it, reversing the sign of the taxis, or altering the intensity of the kinetic component, but little more is known about this subject (review by Arnold 1974). As such, environmental regulation of the rheotropic response may play an important role in the migration of fish. This paper reports the results of experiments designed to quantify the orientational and kinetic components of the rheotropic response of the prespawning common shiner, *Notropis cornutus*, a stream-dwelling migratory species, and to assess the role of temperature and photoperiod in regulating the rheotropic response as a mechanism of upstream migration.

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## Materials and Methods

### OPTOMOTOR TANK

A simple and efficient way of observing the rheotropic response is to simulate the visual stimuli produced by displacement in a water current by moving the background past the fish. Responses to displace-

ment of visual images are specifically referred to as optomotor responses (Arnold 1974). All observations were made in the optomotor tank illustrated in Fig. 1 which was fashioned after that described by Harden Jones (1963). The outer tank was constructed of plywood 121 cm square and 43 cm deep. Into this tank was placed a stainless steel drum attached to a central aluminum core. The opposing surfaces of these two concentric cylinders were painted alternating black and white stripes at intervals of 2.5 cm forming an inner and outer striped background. This assembly was attached to a central shaft and rotated within the outer tank by means of a variable speed reversible motor. The inner stationary tank was constructed of two clear concentric plexiglass rings 43 cm high with an outer diameter of 100 cm and an inner diameter of 20 cm defining a circular channel 40 cm wide. The floor of the channel was featureless and constructed of white plexiglass. The inner tank was suspended from the top of the outer tank such that the enclosed channel was surrounded by the inner and outer rotating striped backgrounds. Water contained within the inner tank was thus separate from water in the outer tank and remained calm despite the rotation of the background. A circular sheet of white plexiglass (not shown in the figure) was attached to the top of the central shaft forming a featureless roof. The inner tank was illuminated from above the roof and surrounded with black curtains. Fish were observed through a peephole in the curtains and their responses to the moving background recorded on a eight-channel event recorder.

Water in the outer tank was circulated through a reservoir equipped with cooling coils and immersion heaters. The floor of the rotating striped drum was perforated to permit free circulation of heated or cooled water around the inner tank providing for accurate temperature control. Water level in the outer tank was set by means of an adjustable standpipe and the inner tank was filled with a water to a depth equal to the level of water in the outer tank. Water depth in the inner tank was maintained at 12 cm for all experiments.

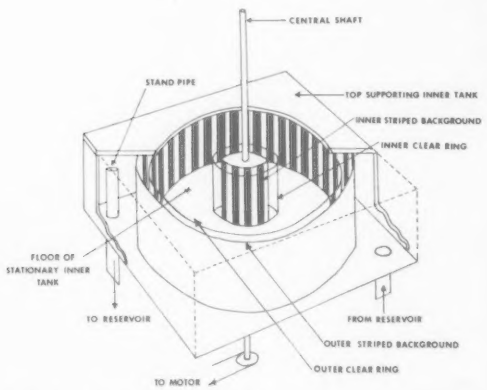


FIG. 1. The optomotor tank used to observe the reaction of common shiners to a moving background. Part of the outer tank is cut away to reveal the outer revolving striped background.

## EXPERIMENTAL PROCEDURE

Common shiners were netted in Laurel Creek on the University of Waterloo campus during their spring upstream spawning migrations in 1975 and 1976. In 1975 fish were captured on May 5 at a water temperature of 12°C and in 1976 on April 17 at a water temperature of 17°C. All fish were held in two closed-system, air-lift stream tanks (described by Gee and Bartnik 1969). Water velocity in the stream tanks was maintained at 10 cm/s. Each stream tank was equipped with lights, cooling coils, and immersion heaters for control of water temperature and photoperiod. In 1975 two groups of 30 fish each and in 1976 two groups of 50 fish each were held in the stream tanks under a day length of 12 and 16 h, respectively, at a water temperature of 10°C for a period of 2 wk prior to the beginning of observations in the optomotor tank. Fish were fed regularly with frozen brine shrimp. Fish examined ranged in fork length from 8.5 to 10.1 cm.

In 1975, 10 fish from each day-length group were observed singly in the optomotor tank at a water temperature of 10°C. The temperature in the optomotor and stream tanks was then raised to 15°C. After an acclimation period of at least 24 h to the new temperature, 10 new fish from each day-length group were observed singly in the optomotor tank. This procedure was repeated for fish observed at 20°C.

In 1976 six groups of 10 fish each were observed under the same conditions of temperature and day-length as described for 1975. Furthermore, the fishes' responses to immediate increases in temperature were observed. Ten fish from each day-length group held at 10°C were observed singly in the optomotor tank at a water temperature of 15°C. Thus, these fish experienced an immediate 5°C increase in water temperature prior to observation. Similarly, 10 fish from each day-length group held at 15°C were observed singly at a water temperature of 20°C.

In 1975 each fish observed in the optomotor tank was allowed 30 min to acclimate to the tank before the background was rotated. Fish were observed for 5 min at each of seven rates of background rotation equivalent to current rates of 2.5, 5, 10, 15, 20, 25, and 30 cm/s (total observation time = 35 min). The direction of background rotation was alternated between fish.

The rheotropic response exhibited by common shiners in 1975 was highly variable. A major source of this variability was the nature of the experimental procedure. Fish exhibited agitated behavior in response to the initial commencement of background rotation and subsequent alteration of background speeds as well as showing signs of fatigue after 35 min of observation. Therefore, each fish observed in 1976 was placed in the optomotor tank with the background revolving at a rate equivalent to a water current of 15 cm/s. The rate of background rotation

was kept constant for the entire observation period which was restricted to 15 min. Each fish was allowed from 5 to 10 min to acclimate to the optomotor tank. Any fish exhibiting excessively agitated behavior or no response at all to the moving background after 10 min of acclimation was not used in the experiment.

### Treatment of Results

The orientational component of the rheotropic response was quantified by recording the proportion of total observation time that fish spent swimming in the same direction as the moving background (frequency of positive rheotaxis). In interpreting the results it must be remembered that swimming in the same direction as the background is equivalent to swimming into a current. The kinetic component of the rheotropic response was quantified by recording the proportion of total observation time a fish spent swimming faster than the moving background (frequency of gain).

The data for the frequency of gain were analyzed by a standard two-way analysis of variance with interaction. The model was of the form:

$$Y_{ijk} = \mu + t_i + p_j + (tp)_{ij} + \epsilon_{ijk}$$

where, $\mu$ is the overall mean; $t_i$ is the effect of the $i$th temperature treatment $i = 1,...,5$; $p_j$ is the effect of the $j$th photoperiod $j = 1,2$; $(tp)_{ij}$ is the photoperiod $\times$ temperature interaction; $Y_{ijk}$ is the observed. frequency of gain for the $k$th ($k = 1,...,10$) fish at the $i$th temperature and the $j$th photoperiod; $\epsilon_{ijk}$ is the experimental error corresponding to $Y_{ijk}$. Prior to detailed analysis, the residuals from the model (the estimates of the $\epsilon_{ijk}$'s) were checked for normality and constant variance. There was no evidence to suggest that these basic assumptions were broken; thus, the experimental error $s^2 = 0.0592$ with 90 df is used throughout the analysis of the kinetic component of the rheotropic response as the best estimate of the common experimental error.

To get a more exact understanding of the significant effects, the Student-Newman-Keuls test and orthogonal contrasts were used.

Data on the frequency of positive rheotaxis were such that neither normality nor constant variance could be assumed and data were split into three categories and analyzed by means of contingency tables.

## Results

### Qualitative Observations

During the 1975 experimental series, common shiners were observed to rest on the bottom of the optomotor tank and exhibit an optokinetic nystagmus (Arnold 1974) at background speeds $< 5$ cm/s. This behavior involved movement of the head and eyes in the same direction and at the same speed as the moving background followed by rapid recovery movements of the head and eyes in the opposite direction. At background speeds $> 5$ cm/s, this behavior was replaced by a full optomotor response in which fish left the bottom of the tank and swam parallel to the moving background. In 1975 four distinct types of optomotor responses were observed.

1) Fish swam round the outer periphery of the tank in the same direction as the moving background (positive rheotaxis). The proportion of time fish spent swimming faster than the background was similar at each rate of background rotation but this frequency differed widely between individual fish.

2) Fish swam round the outer periphery of the tank in the opposite direction to the moving background (negative rheotaxis).

3) Fish swam rapidly back and forth over short distances at the outer periphery in an apparent attempt to intercept the moving stripes.

4) Fish swam tail-first round the outer periphery in the same direction as the moving background. This unusual behavior involved flexing the caudal fin far to one side and short strokes of the pectoral fins. The more powerful strokes of the caudal and pectoral fins were towards the head. This response generally did not persist at background speeds $> 10$ cm/s.

Individual fish tested in 1975 exhibited all four optomotor responses in widely varying proportions.

During the 1976 experimental series common shiners exhibited positive rheotaxis immediately upon being introduced into the optomotor tank with the background revolving constantly at 15 cm/s. Negative rheotaxis was rarely observed and optomotor responses (3) and (4) were not observed.

The pattern of swimming exhibited by common shiners in both years varied with temperature. At 10°C fish swam close to the bottom of the tank and maintained the same depth while swimming round the tank. At 20°C fish swam from the surface to the bottom of the tank in sine wave fashion never maintaining the same depth while swimming round the tank. Both patterns of swimming were observed at 15°C.

The 1975 experimental series indicated that the frequency of positive rheotaxis and the frequency of gain exhibited by common shiners varied according to temperature and day length. However, both of these frequencies were highly variable among fish of the same experimental group. As a result, differences between groups were obscured and no definite conclusions could be reached regarding the nature and extent of the regulation of rheotropism by temperature and photoperiod.

### Quantitative Results

The frequency of positive rheotaxis and the frequency of gain did not vary as greatly among fish

of the same experimental group in 1976 as in 1975. This reduction in within-group variation permitted a complete quantitative analysis of the 1976 data. These results are summarized in Table 1. The analyses of the orientational and kinetic components of the rheotropic response are dealt with below.

*Kinetic component* — The significant sources of variation between observed differences in the frequency of gain were temperature ($F = 13.0$, $P < 0.001$) and an interaction between temperature and photoperiod ($F = 3.3$, $P < 0.01$).

Variation in mean frequency of gain was as follows:

| acclimated 24 h | | | immediate 5°C increase | |
|---|---|---|---|---|
| 10°C | 15°C | 20°C | 15°C | 20°C |
| 0.117 | 0.194 | 0.517 | 0.475 | 0.532 |

Mean frequency of gain of fish acclimated 24 h to 10 and 15°C were significantly less than the remainder ($P < 0.05$, Student-Newman-Keuls test). Thus, fish observed at 15°C following 24 h of acclimation exhibited a lower frequency of gain than fish observed at 15°C following an immediate 5°C increase in water temperature. Fish observed at 20°C exhibited a similar frequency of gain regardless of acclimation time. The frequency of gain exhibited by fish observed at 20°C after 24 h of acclimation was significantly greater than the frequency of gain observed at 10 and 15°C after 24 h of acclimation.

The variation in frequency of gain due to the interaction of temperature and photoperiod was analyzed by means of orthogonal linear contrasts. In this case, the first contrast amounted to a comparison of the regressions of frequency of gain on the three temperatures of acclimation for each photoperiod. The contrast showed that the slopes of the two regression lines ($y = -0.583 + 0.058T$ for 12-h day length; $y = -0.065 + 0.022T$ for 16-h day length) were significantly different ($t = 2.34$, $P < 0.02$) indicating a greater increase in the frequency of gain with temperature of acclimation for fish exposed to 12 h of daylight than for fish exposed to 16 h of daylight. This difference is responsible for nearly 50% of the interaction variation. There was no statistical evidence of a significant deviation from linearity in the two regressions.

Another orthogonal contrast revealed that most of the remainder of the interaction variation was due to the differential response to immediate increases in temperature exhibited by fish held under the two photoperiods. Fish exhibited a similar frequency of gain in response to a water temperature of 15°C following an immediate 5°C increase regardless of day length (0.45 for 12-h day length and 0.50 for 16-h day length). However, fish exposed to a 12-h day length exhibited a significantly greater frequency of gain in response to a water temperature of 20°C following an immediate 5°C increase than did fish exposed to a 16-h day length (0.70 for 12-h day length and 0.36 for 16-h day length; $P < 0.02$).

TABLE 1. The effect of water temperature and day length on the orientational and kinetic components of the rheotropic response of the common shiner, *Notropis cornutus*. $N = 10$; frequency of positive rheotaxis = proportion of 15-min observation time fish spent swimming in the same direction as the moving background; frequency of gain = proportion of 15-min observation time fish spent gaining on the moving background; *fish were acclimated at least 24 h at that temperature; **fish were observed following an immediate 5°C increase in water temperature.

| Day length (*h*) | Temp. (°C) | Mean frequency +ve rheotaxis | Mean frequency gain |
|---|---|---|---|
| 12 | 10* | 0.98 | 0.05 |
| | 15** | 0.95 | 0.45 |
| | 15* | 0.98 | 0.18 |
| | 20** | 0.95 | 0.70 |
| | 20* | 0.80 | 0.63 |
| 16 | 10* | 0.97 | 0.18 |
| | 15** | 0.99 | 0.50 |
| | 15* | 0.99 | 0.20 |
| | 20** | 0.90 | 0.36 |
| | 20* | 0.93 | 0.40 |

*Orientational component* — The number of common shiners exhibiting positive rheotaxis 100% of the time, between 90 and 99% of the time, and < 90% of the time at two photoperiods and five temperature conditions is presented in Table 2. Photoperiod was not a significant source of variation between the number of fish observed within each frequency category ($\chi^2 = 1.09$, df = 2, $P > 0.05$), whereas temperature was a significant source of variation ($\chi^2 = 40.22$, df = 8, $P < 0.001$). In particular, fish observed at 20°C following 24 h of acclimation exhibited lower frequencies of positive rheotaxis than fish observed at other temperatures.

## Discussion

This study demonstrates that the response of common shiners to a simulated water current is affected by water temperature and photoperiod. Interpreting the experimental variable of frequency of gain as a measure of the frequency

TABLE 2. The number of common shiners exhibiting positive rheotaxis as percent of 15-min observation time at five temperature conditions; *fish acclimated at least 24 h at that temperature; **fish observed following an immediate 5°C increase in water temperature.

| Frequency of +ve rheotaxis (% observation time) | No. fish observed at (°C) | | | | |
|---|---|---|---|---|---|
| | 10* | 15* | 20* | 15** | 20** |
| Fish exposed to 12-h day length | | | | | |
| 100 | 6 | 8 | 0 | 5 | 4 |
| 90–99 | 4 | 1 | 2 | 4 | 4 |
| <90 | 0 | 1 | 8 | 1 | 2 |
| Fish exposed to 16-h day length | | | | | |
| 100 | 8 | 7 | 1 | 6 | 2 |
| 90–99 | 0 | 3 | 5 | 4 | 6 |
| <90 | 2 | 0 | 4 | 0 | 2 |

of upstream movement, the combination of spring day length (12 h) and warm water temperatures (20°C) induced the greatest frequency of upstream movement in the common shiner. Further increases in the frequency of upstream movement followed rapid 5°C increases in water temperature in combination with spring and summer day lengths (Table 1), but most consistently in combination with spring day lengths. Interpreting the experimental variable of frequency of positive rheotaxis as a measure of the frequency of upstream orientation, neither temperature nor photoperiod had a major effect on the frequency of upstream orientation. Although the frequency of this behavior decreased at 20°C in combination with spring and summer day lengths, common shiners continued to exhibit upstream orientation at least 80% of the time (Table 1).

Water temperature appears to be an important factor affecting the rheotropic response of juveniles and adults of several species of fish. Keenleyside and Hoar (1954) studied the rheotactic responses of three species of juvenile Pacific salmon (genus *Oncorhynchus*) under varying temperature conditions and demonstrated that rheotaxis was predominantly positive at lower temperatures but became actively negative at higher temperatures. Northcote (1962) concluded that temperature played a predominant role in controlling the rheotropic response of juvenile rainbow trout, *Salmo gairdneri*, migrating into Loon Lake. Of particular interest is the observation that fingerlings subjected to rapid 5°C increases in water temperature exhibited immediate increases in upstream movement. The evidence concerning the influence of temperature on upstream movement in adult salmonids is extensive and conflicting (review by Banks 1969). Lindsey and Northcote (1963) reported that the time of first entrance of adult redside shiners, *Richardsonius balteatus*, into spawning streams showed a close correlation with the date at which creek temperatures first exceeded 10°C. Throughout the spawning season, surges of upstream migration were associated with rising water temperatures. MacLean and Gee (1971) have suggested that a positive rheotactic response in association with water temperatures between 15 and 20°C may be the mechanism responsible for the observed migration of prespawning brook sticklebacks, *Culaea inconstans*, into the shallow warm meltwater ponds and ditches where they reproduce. This evidence and that provided by the present study suggest that water temperature is a common factor controlling upstream movements in several species of fish with varying orientational requirements.

The role of photoperiod in regulating the rheotropic response is not well documented. Northcote (1958) demonstrated that fingerling rainbow trout held at a 16-h day length exhibited a strong negative rheotaxis, particularly at low water temperatures, whereas 8-h day length fingerlings exhibited positive rheotaxis at low water temperatures. The results of this study demonstrate that the interaction of photoperiod and temperature regulates the frequency of upstream movement of common shiners. Thus, the mechanism of upstream migration is complex and not regulated only by solitary environmental factors.

A common observation in many studies of rheotropism in fishes as revealed by their reactions to moving backgrounds is the occurrence of wide variations in the optomotor reactions of individual fish of the same species. A number of reasons have been presented to explain this variation. The present study has demonstrated that temperature and photoperiod are two important sources of such variation. Clausen (1931) examined the optomotor reactions of the common

shiner and observed positive rheotaxis, negative rheotaxis, and "tail-first" swimming. Clausen noted an apparent stimulatory effect of the rotation of the background on the occurrence of negative rheotaxis regardless of whether the background was moved quickly or slowly. The results of the 1975 experimental series indicate a similar relationship. Negative rheotaxis, as well as optomotor reaction (3) (see Qualitative Observations), was observed frequently in response to the alteration of the rate of background rotation but independent of the magnitude of rate. In 1976 the rate of background rotation was held constant and common shiners exhibited positive rheotaxis most of the time. As such, alteration of the rate of background rotation may in itself be an important source of variation between the optomotor reactions of individual fish.

The upstream movements of common shiners in this study conform to the general pattern of migratory activity exhibited by common shiners in nature. An inshore migration in lakes and an upstream movement in streams are made by common shiners in early May when the water has reached a temperature of 12–15.5°C (55–60°F) (Raney 1940). Spawning usually begins when water temperatures reach 15.5–18°C (60–65°F) usually in May or June (Raney 1940). The present study has revealed that temperature is more important than photoperiod in controlling upstream migration. This conclusion is supported by the observation that in 1976 shiners were captured in mid-April when water temperatures were already at 17°C due to an early spring heat wave. Water temperatures subsequently declined to 10°C but attempts to capture shiners in May and June failed, a probable indication that spawning had already been completed.

The regulation of the rheotropic response of common shiners by the nondirectional environmental factors of temperature and photoperiod forms the basis of a mechanism of upstream migration that ensures shiners arrive on the spawning site at a time that corresponds with the required spawning temperature. Such a mechanism is of particular adaptive significance in small streams that may be subject to rapid fluctuations in water temperature early in the spring.

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# Hydrocarbons in Eastern Canadian Marine Waters Determined by Fluorescence Spectroscopy and Gas–Liquid Chromatography[1]

P. D. KEIZER, D. C. GORDON JR., AND J. DALE

*Department of Fisheries and the Environment, Fisheries and Marine Service, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2*

KEIZER, P. D., D. C. GORDON JR., AND J. DALE. 1977. Hydrocarbons in eastern Canadian marine waters determined by fluorescence spectroscopy and gas–liquid chromatography. J. Fish. Res. Board Can. 34: 347–353.

Concentrations of *n*-alkanes ranged from <20 ng/ℓ to about 1 μg/ℓ at depths of 2 and 50 m. Estimated oil concentrations based on fluorescence analyses ranged from 0.2 to 9.3 μg/ℓ. The highest concentrations occurred in Bedford Basin; the similarity of fluorescence contour plots to petroleum products and the presence of unresolved envelopes in chromatograms suggest that a large portion of the hydrocarbons in the Basin are derived from oil. Hydrocarbons in other regions appear to be principally biogenic in origin.

*Key words*: Oil detection, northwest Atlantic, gas–liquid chromatography, fluorescence spectroscopy

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Les concentrations de n'alcanes varient de < 20 ng/ℓ à environ 1 μg/ℓ aux profondeurs de 2 et 50 m. Les concentrations de pétrole, telles qu'estimées par analyses par fluorescence, vont de 0.2 à 9.3 μg/ℓ. Les plus fortes concentrations se trouvent dans le bassin de Bedford; la similitude des tracés de contours de fluorescence avec ceux des produits pétroliers et la présence d'enveloppes non résolues dans les chromatogrammes donnent à croire qu'une forte portion des hydrocarbures présents dans le Bassin sont dérivés du pétrole. Les hydrocarbures des autres régions semblent être d'origine surtout biogénique.

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As a result of the *Arrow* disaster in Chedabucto Bay, N.S., during February 1970 and considerable oil exploration activity, numerous surveys have been made of hydrocarbons in the marine environment off Atlantic Canada. The principal analytical method used, for sea water and sediments, has been fluorescence spectroscopy (for example Levy 1971; Keizer and Gordon 1973; Gordon et al. 1974; Hargrave and Phillips, 1975).

Being a complex mixture of hydrocarbons, oil in the marine environment can be detected by numerous analytical methods. Fluorescence spectroscopy detects only those compounds that fluoresce, principally the aromatic hydrocarbons and their oxygenated derivatives. To learn more about the occurrence of aliphatic hydrocarbons, we have adopted procedures for analyzing environmental samples by gas–liquid chromatography.

Since individual hydrocarbons are being detected and concentrations are usually in the namograms per litre range, large volumes of sea water (10–100 ℓ) must be collected and processed. To avoid the disadvantages of liquid–liquid extractions of large seawater volumes, we explored the possibility of using a macroreticular resin to extract hydrophobic hydrocarbons; sorption of hydrocarbons to the sampling equipment and serious unsolved contamination problems, however, forced us to revert to liquid–liquid extractions. Recognition of the magnitude of the sorption problem led to the development of a new sampler for collecting noncontaminated sea water at depths down to at least 50 m.

In this paper, we describe our sampling and analytical techniques and present some recent data on hydrocarbon concentrations in sea water off Atlantic Canada.

## Materials and Methods

Uncontaminated sea water was collected at 2 and 50 m at stations shown in Fig. 1. The 2-m samples

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FIG. 1. Sampling locations for the Scotian Shelf (01) Gulf of St. Lawrence (06, 53, 30), and St. Lawrence estuary (41, 46) during May 1975 on Cruise 75-015 of the C.S.S. *Dawson*. Bedford Basin samples (BB) were collected between January and May 1975 at the Compass Buoy which is located near the center in 70 m of waters. Sargasso Sea samples (SS) were collected during February 1975 at 26°00'N, 62°40'W on Cruise 75-004 of the C.S.S. *Dawson*.

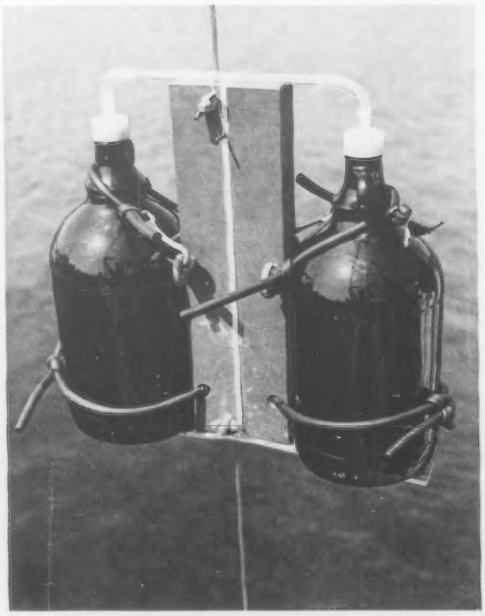

FIG. 2. Water sampler attached to hydrographic wire ready for lowering. The sampler is subsequently activated by dropping a messenger. Maximum depth is about 70 m.

were collected with an apparatus described by Gordon and Keizer (1974). It consists of a 4-$\ell$ glass bottle (which can easily be interchanged between casts) held in a weighted metal frame; the whole device is attached to a line and thrown about 10 m away from the ship. A float suspends the bottle at the proper depths while it fills. The glass bottles were cleaned and rinsed well with doubly distilled pentane before sampling.

The 50-m samples were collected with a modified in situ sampler described by Watt (1965). Two 4-$\ell$ glass reagent bottles are attached to a PVC and aluminum frame (Fig. 2). The bottles are sealed with Teflon® screw caps which are connected with two elbows of Tygon tubing and a glass tube. The latter is held close to the wire with an elastic band. After the sampler is lowered to the sampling depth (the glass bottles generally implode at about 70 m), a messenger is dropped which breaks the glass tube and water is drawn into the bottles. Approximately 3 min is required to fill the bottles at 50 m. Glass bottles, Teflon® caps, and Tygon and glass tubing are rinsed well with doubly distilled pentane before the sampler is assembled. Before the sampler is used routinely on a specific vessel, checks must be made to insure that the hydrographic wire is not a source of contaminating hydrocarbons. If it is, a substitute such as polypropylene line should be used.

At each station, 36 $\ell$ of sea water were collected at 2 and 50 m; then the bottles were arbitrarily divided into three lots. Each lot (12 $\ell$) constituted a sample and was handled and analyzed separately. The unfiltered sea water was extracted twice within 4 h with doubly distilled pentane (100 ml/3 $\ell$ sea water). Sample bottles were well rinsed with additional pentane to remove sorbed hydrocarbons and the rinse was added to the extracts. Extracts from Bedford Basin were processed immediately, while those from other areas were stored in a freezer until returned to the laboratory.

Water was carefully removed from the extracts in small separatory funnels and a small amount of anhydrous sodium sulfate was added to remove any residual water. The pentane extract was decanted into a Kuderna-Danish concentrator and concentrated to 10.0 ml. At this stage most extracts were analyzed by fluorescence spectroscopy (Gordon and Keizer 1974) using a Perkin-Elmer MPF-2A instrument. Concentrations of "oil" were estimated with Guanipa crude oil as a standard (excitation at 310 nm, emission at 374 nm). Emission at various wavelengths (excitation and emission) was also measured and fluorescence contour plots were generated with an off-line plotting routine (General Purpose Contouring Program, Calcomp Corp.) using a PDP8 computer to drive a Calcomp 563 Plotter (Hargrave and Phillips 1975).

After further concentration to 0.5 ml, the extracts were placed on prerinsed columns of alumina (activated at 400°C for 16 h, deactivated 5% with water) on silica gel (activated at 250°C for 16 h, deactivated 5% with water) in 60- × 3-mm glass columns and the saturated fractions eluted with 3.5 ml of pentane.

TABLE 1. Range of *n*-alkane (GLC) and estimated oil (fluorescence) concentrations in the five geographic regions sampled. Sampling dates and exact locations are given in Fig. 1. ND = not detectable.

| Region | *n*-alkanes (ng/ℓ) | | Estimated oil (μg/ℓ) | |
|---|---|---|---|---|
| | 2m | 50m | 2m | 50m |
| Bedford Basin | 65–590 | 25–395 | 1.6–9.3 | 1.4–4.3 |
| Scotian Shelf | 78–99 | 80–154 | 0.2–0.8 | 0.2–2.0 |
| Gulf of St. Lawrence | 24–209 | 76–1005 | 0.6–1.1 | 0.5–1.5 |
| St. Lawrence estuary | 34–96 | 137–372 | 0.5–1.1 | 0.4–0.9 |
| Sargasso Sea | ND–118 | 54–145 | — | — |

The eluate was concentrated under a stream of purified nitrogen to 100 μl. Twenty-microlitre aliquots were placed in aluminum capsules and evaporated almost to dryness folowing which the capsules were sealed by a cold weld.

The samples were injected using an MS-41 sampling accessory on a Perkin-Elmer 3920 flame-ionization gas chromatograph fitted with a 3 m × 0.3 cm OD stainless steel column loaded with 60/80 mesh Chromosorb W(AW) coated with 3% Apiezon L. The column was programmed from 80 to 280°C at 8°C/min. Injector temperature was 250°C and the detector temperature, 300°C. The pristane–*n*-heptadecane peaks were adequately separated for quantitation but the phytane–*n*-octadecane peaks were not. Compounds were identified by comparison of retention times with those of pure *n*-alkane standards obtained from Applied Science. Calculations were based on peak areas using an external standard. *N*-alkanes from *n*-tetradecane ($C_{14}$) to *n*-heptacosane ($C_{27}$) were quantified along with pristane and any other major peaks.

Two blanks were prepared with each set of samples by rinsing sample bottles and separatory funnels (after extraction) with 100 ml pentane. The rinse was subsequently treated as a sample. Typically, blanks were < 10% of sample concentrations for gas chromatography and < 5% for fluorescence. Test recoveries of spiked samples and exhaustive extraction of natural samples gave recoveries > 80% for saturated hydrocarbons with boiling points equal to or greater than that of *n*-pentadecane ($C_{15}$). Values reported are not corrected for losses during the extraction procedure since this factor is small compared with variations due to sampling.

Since each method is detecting different components of the total hydrocarbon assemblage in sea water, differing results should be expected. The fluorescence method supplies an estimate of an "oil" concentration based on the fluorescence of aromatic hydrocarbons and some arbitrary standard. Gas chromatography is much more exacting and provides accurate concentrations of individual saturated hydrocarbons. However, if the saturated and aromatic hydrocarbons are of the same source, a correlation between concentrations may occur. This did occur in those samples collected at 2 m in Bedford Basin ($n = 21$, $r = 0.511$, $P < 0.01$) suggesting the presence of petroleum-derived hydrocarbons.

## Results

### BEDFORD BASIN

Concentrations of *n*-alkanes in Bedford Basin varied from 65 to 590 ng/ℓ at 2 m and from 25 to 395 ng/ℓ at 50 m between January and May 1975 (Table 1). On all nine sampling dates, the concentrations in replicates varied considerably (Fig. 3). Concentrations at 2 m were significantly greater than those at 50 m ($n = 51$, $t = 2.21$, $P < 0.05$).

Qualitatively, the patterns of *n*-alkane peaks at both depths were very similar (Fig. 4a). The most abundant alkanes were generally *n*-heptadecane ($C_{17}$) and *n*-octadecane ($C_{18}$); the least abundant generally being *n*-eicosane ($C_{20}$) and *n*-heneicosane ($C_{21}$). In contrast, the patterns of non-*n*-alkane peaks were quite variable. The concentration of pristane was occasionally high (at both depths) and large peaks with retention indices of 2020 and 2060 were observed in a number of samples (no correlation with pristane). The 2020 peak could well be the $C_{21}$ hexaene identified by Blumer et al. (1970), which occurs in plankton. Since the sea water was not filtered before extraction, phytoplankton and perhaps some zooplankton were present. An unresolved envelope was present in most samples (Fig. 4a) and ranged from very small to very large.

Estimated oil concentrations, using fluorescence spectroscopy, varied from 1.6 to 9.3 μg/ℓ at 2 m and from 1.4 to 4.3 μg/ℓ at 50 m. The range of replicates was again considerable (Fig. 5). Concentrations at 2 m were significantly greater than at 50 m. However, there was no apparent different in fluorescence contour plots with depth. Fluorescence emission is maximal at an excitation wavelength of approximately 300 nm and diminishes gradually, a pattern typical of petroleum-derived products (Fig. 6a).

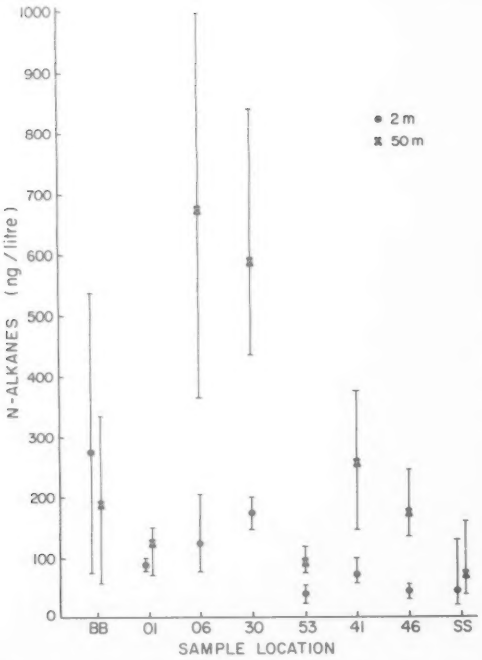


FIG. 3. Range and mean of *n*-alkane concentrations (GLC) at all regions sampled (Fig. 1). The Bedford Basin (BB) data represent nine separate stations; remaining data represent single stations.

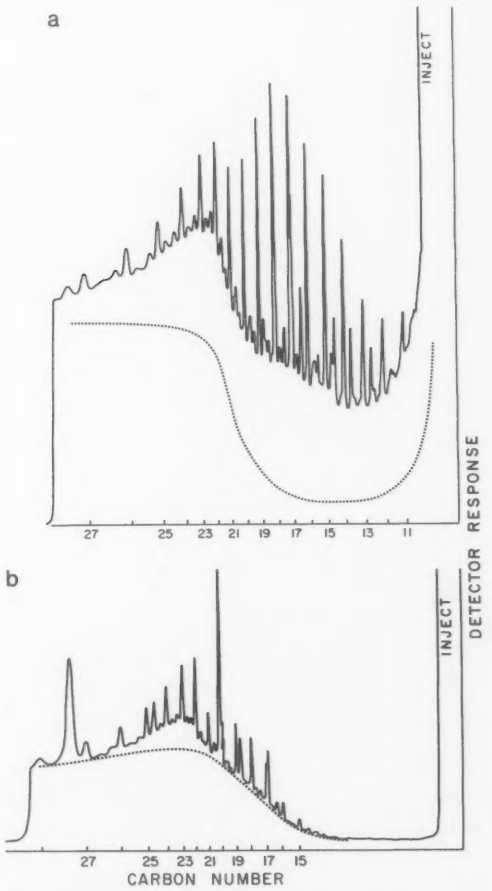


FIG. 4. Traces of actual chromatograms of seawater extracts; a, Bedford Basin and b, Gulf of St. Lawrence. The dashed line represents the baseline; the area between it and the peaks represents the unresolved envelope.

## SCOTIAN SHELF

The concentration of *n*-alkanes at the single station sampled ranged from 78 to 99 ng/$\ell$ at 2 m and 80 to 154 ng/$\ell$ at 50 m (Table 1, Fig. 3). The *n*-alkane patterns were similar to those of the Bedford Basin samples but the unresolved envelope was negligible. High pristane concentrations were observed at both depths as was a large peak with a retention index of 2020.

The estimated oil concentrations were moderately variable (Table 1, Fig. 5). The fluorescence contour plots were quite unusual and unlike those from Bedford Basin or from any of the petroleum products analyzed in our laboratory.

## GULF OF ST. LAWRENCE

This area includes Stations 06, 30, and 53 (Fig. 1). Concentrations of *n*-alkanes in the 2-m samples ranged from 24 to 209 ng/$\ell$, while in the 50-m samples they ranged from 76 to 1005 ng/$\ell$. We did not detect higher concentrations in Cabot Strait or near the Irving Whale as observed by Levy and Walton (1973) in 1971. Concentrations at Station 53 were much lower at both depths than at the other two locations. At all three locations, concentrations at 50 m were significantly higher. Again, the *n*-alkane patterns were similar to those observed in Bedford Basin (Fig. 4b). Unresolved envelopes were negligible except at Cabot Strait, particularly at 50 m. A large peak with a retention index of 2060 was observed in all 50 m samples.

Estimated oil concentrations were low and showed little variation (Table 1, Fig. 5). Off the Gaspé coast, concentrations at 50 m were greater than at 2 m. Due to the low concentrations the fluorescence contour plots had poor detail but

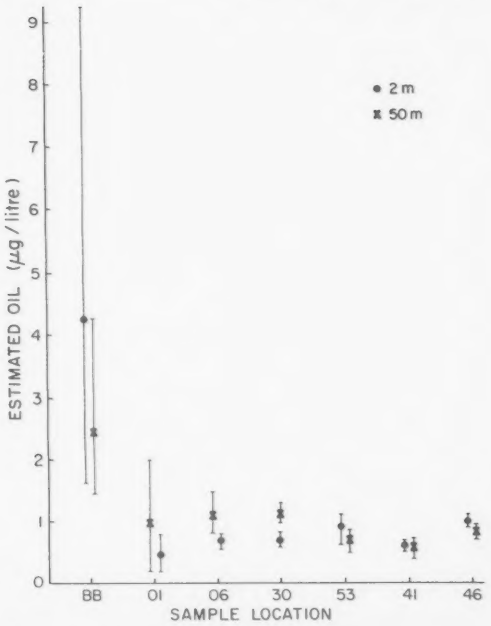


FIG. 5. Range and mean of estimated oil concentrations (fluorescence) at all regions sampled (Fig. 1) except Sargasso Sea (where Gordon et al. 1974 report concentrations $< 1$ $\mu g/\ell$). The Bedford Basin (BB) data represent seven separate stations; remaining data represent single stations.

despite this some unique features were apparent (Fig. 6b); the nature of the fluorescing material from the Gulf was quite different from that in the Bedford Basin (Fig. 6a).

### ST. LAWRENCE ESTUARY

The concentrations of *n*-alkanes tended to be lower than in the Gulf but like the Gulf the concentrations at 50 m were greater than at 2 m (Table 1, Fig. 3). The *n*-alkane patterns were similar to those observed in all other regions, and there was no appreciable unresolved envelope.

Estimated oil concentrations were among the lowest observed (Table 1, Fig. 5) and variability was slight. Fluorescence contour plots were similar to those observed in the Gulf (Fig. 6b).

### SARGASSO SEA

The Sargasso Sea samples were analyzed for *n*-alkanes only. Three of the four 2-m samples had concentrations less than the blank (<20 ng/$\ell$). Concentrations at 50 m ranged from 54 to 145 ng/$\ell$ (Table 1, Fig. 3). Chromatograms were

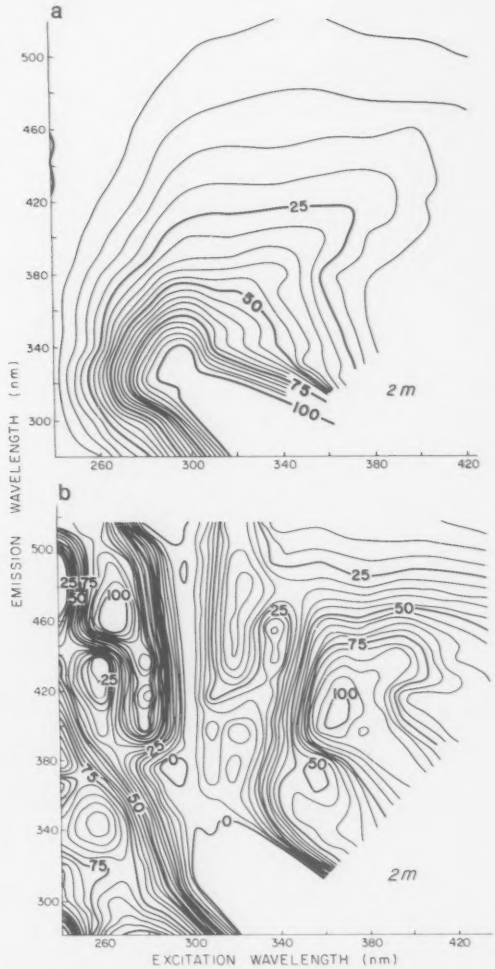


FIG. 6. Typical fluorescence contour plots of sea water (2-m depth) from a. Bedford Basin, and b. the Gulf of St. Lawrence.

similar to others except those from Bedford Basin. Concentrations determined by fluorescence analysis of other samples collected in this area (Gordon et al. 1974) were $< 1$ $\mu g/\ell$.

## Discussion

Oil concentrations reported here are in the same range as those reported for Scottish coastal waters (210–3100 ng/$\ell$) and the Gulf of Mexico (28–1100 ng/$\ell$) by Mackie et al. (1974) and Parker et al. (1972), respectively, using similar analytical methods. Concentrations reported from the Gulf

of Mexico and Caribbean Sea by Iliffe and Calder (1974) and from coastal waters of France and West Africa by Barbier et al. (1973) are not comparable because they represent the total saturate fraction which was determined gravimetrically.

The pattern of *n*-alkane peaks is generally similar to that observed in sea water by other workers in different regions. Minor qualitative variations can be observed but these can be explained by differences in analytical procedure (solvent, chromatographic column, etc.).

Due to contamination picked up from the surface film and water column, conventional oceanographic sampling bottles, which are lowered open, are not suitable for collecting sea water for hydrocarbon analysis (Gordon et al. 1974). The only suitable samplers for this application are those whose inner surface contacts only the water being sampled and which can be rinsed with organic solvent to recover sorbed hydrocarbons after each use. The only commercially suitable sampler possessing these features is the Blumer bottle (manufactured by Benthos Inc.). It is expensive, however, and difficult to handle. Another alternative for collecting contamination-free sea water is to supply divers with glass bottles that can be opened and sealed at the desired sampling depth (Mackie et al. 1974). The sampling device that we describe in this paper also appears suitable for collecting sea water relatively free of contamination and has the benefits of being inexpensive to construct (materials less than $100) and easy to operate (only one person and a winch required). If necessary, the depth range could be increased by using spherical glass flasks.

The similarity of the Bedford Basin fluorescence contour plots to those of oil standards indicates that the aromatic compounds present were derived mainly from petroleum. Conceivably, some of the *n*-alkanes could have had the same origin, especially at 2 m, where the strong correlation existed. Fluorescence contour plots from the other regions sampled bore no resemblance to oil and suggest that the hydrocarbons detected were principally biogenic. Therefore, the only location sampled with detectable levels of oil pollution in the water column is Bedford Basin. Estimated oil concentrations from other regions are probably meaningless for the aromatic compounds detected by fluorescence were apparently not derived from petroleum.

The 2-m samples in Bedford Basin had significantly greater concentrations of *n*-alkanes than 50-m samples. The opposite was true in the gulf and estuary of the St. Lawrence (Fig. 3). A similar distribution is observed in particulate organic carbon concentrations (R. Pocklington, personal communication, Bedford Institute of Oceanography; our own unpublished data) which suggests some type of relationship, perhaps indirect, between *n*-alkane and particulate organic matter concentrations. This apparent relationship is not surprising. Since most of the *n*-alkanes observed appear to be biogenic in origin, it is reasonable to find the greatest concentrations at or just below the depth of maximum biological activity. Also, the low solubility of *n*-alkanes is well documented and experimental evidence indicates that a large fraction of petroleum hydrocarbons entering sea water is accommodated in a particulate form (Boehm and Quinn 1974). More precise information regarding the form of hydrocarbons occurring in sea water, especially at low concentrations, is required. Recent work by Daumas et al. (1976) suggests that appreciable concentrations of dissolved alkanes can occur and be qualitatively much different from particulate alkanes.

The standard deviation observed in the fluorescence spectroscopy data was much less than in Gordon et al. (1974). This can be attributed to the larger sample volume (12 instead of 2 $\ell$) which masks much of the small scale patchiness in the distribution of fluorescing compounds.

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# Estimates of Tag Extrusion and Initial Tagging Mortality in Atlantic Herring (*Clupea harengus harengus*) Released with Abdominally Inserted Magnetic Tags

G. H. WINTERS

*Department of Fisheries and the Environment, Fisheries and Marine Service, Biological Station, St. John's, Nfld. A1C 1A1*

WINTERS, G. H. 1977. Estimates of tag extrusion and initial tagging mortality in Atlantic herring (*Clupea harengus harengus*) released with abdominally inserted magnetic tags. J. Fish. Res. Board Can. 34: 354–359.

Returns of Atlantic herring (*Clupea harengus harengus*) tagged internally with magnetic tags along southwest Newfoundland and in the southern Gulf of St. Lawrence in 1970–71 were analyzed for biases due to initial tagging mortality and to tag extrusion during the reproductive act. Initial tagging mortalities calculated by two methods ranged from 4 to 49% and increased with the duration of impoundment of the fish prior to tagging. Estimates of tag loss during the spawning season indicated that most of the tags inserted in ripening herring were extruded with the reproductive products, whereas those applied to recently spent or recovering herring had sufficient time to become attached to internal organs and hence had a high retention rate. The results indicate that the effectiveness of tagging herring internally will be greatly improved if conducted on recovering or spent fish impounded for only short periods of time before tagging.

WINTERS, G. H. 1977. Estimates of tag extrusion and initial tagging mortality in Atlantic herring (*Clupea harengus harengus*) released with abdominally inserted magnetic tags. J. Fish. Res. Board Can. 34: 354–359.

Nous avons analysé les recaptures de harengs atlantiques (*Clupea harengus harengus*) marqués d'étiquettes magnétiques internes le long de la côte sud-ouest de Terre-Neuve et dans le golfe Saint-Laurent méridional en 1970–71, dans le but de détecter des erreurs systématiques résultant de la mortalité initialé initiale à l'étiquettage et de l'extrusion de la marque au moment de la fraie. Les mortalités initiales après l'étiquettage, calculées selon deux méthodes, varient de 4 à 49% et augmentent en fonction de la durée de retenue des poissons avant l'étiquettage. Des estimés de perte d'étiquettes pendant la fraie indiquent que la plupart des étiquettes introduites dans des harengs en voie de maturation sont expulsées en même temps que les produits sexuels, alors que celles qui sont introduites dans des harengs ayant récemment frayé ou en voie de rétablissement ont suffisamment de temps pour se fixer aux organes internes et ont, par conséquent, un taux de rétention élevé. Les résultats indiquent qu'on améliorerait de beaucoup l'efficacité de l'étiquettage interne du hareng si on le faisait sur des harengs vides et en voie de rétablissement, et maintenus en captivité pendant de courtes périodes seulement avant l'étiquettage.

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IN addition to their use for determining migration patterns of fish, mark–recapture experiments may also be used for estimation of certain population parameters, particularly stock size and exploitation rate (Ricker 1958). Estimates of these parameters may be affected by systematic errors in the rate of tag recaptures due, for example, to extra mortality amongst marked fish resulting from the effects of the tag and its application (Ricker's Type A error), or to a sudden loss of tags (a variant of Ricker's Type B error). In such cases, valid parameter estimation requires some adjustment of recapture data.

During the early 1970s extensive tagging experiments were conducted (Winters 1971) to test the hypothesis of Hodder (1966, 1969) that the herring fisheries which developed along southwestern Newfoundland (Area J, Fig. 1) and in the southern Gulf of St. Lawrence (Area T, Fig. 1) during the mid-1960s were not based so much on different stocks as on parts of the same stock complex at different times and places along its seasonal migration route. Complementary tagging studies were conducted in the southern Gulf (Beckett 1971). This communication documents

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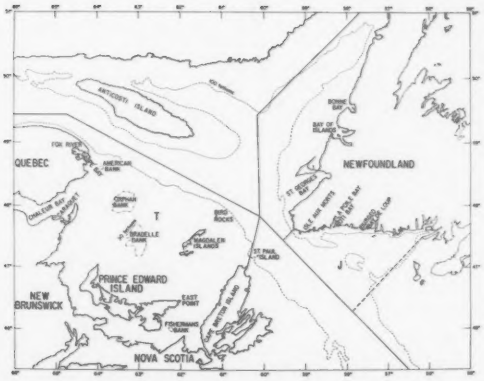


FIG. 1. Map of southern Gulf of St. Lawrence (Area T) and southwest Newfoundland (Area J).

the occurrences of Type A and B errors in these tagging experiments and describes several techniques by which such biases were evaluated and quantified.

## Materials and Methods

The nature of the herring fisheries in Areas T and J (bulk catches processed for meal and oil) necessitated the use of internal magnetic tags (19 × 4 × 1 mm) as the main method of marking; these were to be recovered by magnetic separators normally installed in the processing lines of reduction plants.

Live Atlantic herring (*Clupea harengus harengus*) were captured with a bar-seine 160 m long and 16 m deep (Winters 1975). An average bar-seine set could generally supply enough fish for 2–3 days tagging operations. Herring were barred within small coves until required for tagging at which time appropriate quantities (5,000–10,000 fish) were transferred to holding pounds 5 m deep and 6 m on each side. Tagging took place in small boats secured to the side of these holding pounds from which live herring were dipped into canvas or plastic tagging tanks capable of holding 50–75 herring each. Herring were removed individually from the tanks and a small incision was made in the belly through which the magnetic tag was inserted and pushed forward into the abdominal cavity. Before being released the tagged herring was flexed seevral times to ensure that the tag was completely inside the body cavity.

A summary of the tagging carried out along southwest Newfoundland and subsequent recapture is given in Table 1.

TABLE 1. Summary of recapture data by liberation series and day of release for the 1970 and 1971 tagging experiments along southwest Newfoundland.

| Liberation series | No. tagged | Recaptures | |
|---|---|---|---|
| | | *N* | % |
| A1 | | | |
| Mar. 3/70 | 1,100 | 44 | 4.00 |
| 4/70 | 4,600 | 157 | 3.41 |
| 5/70 | 2,700 | 49 | 1.81 |
| Total | 8,400 | 250 | 2.98 |
| A2 | | | |
| Mar. 7/70 | 4,500 | 113 | 2.51 |
| 8/70 | 300 | 2 | 0.67 |
| Total | 4,800 | 115 | 2.40 |
| A3 | | | |
| Mar. 11/70 | 2,000 | 120 | 6.00 |
| 12/70 | 7,000 | 239 | 3.41 |
| 13/70 | 2,800 | 30 | 1.07 |
| Total | 11,800 | 389 | 3.30 |
| Grand Total A | 25,000 | 768[a] | 3.07 |
| B | | | |
| Jan. 25/71 | 4,200 | 389 | 9.26 |
| 26/71 | 5,800 | 77 | 1.33 |
| Grand Total B | 10,000 | 466 | 4.66 |

[a]Includes 14 tags of unknown tag number.

## Results

### Estimation of Initial Tagging Mortality and Effective Number Tagged

Examination of tagged fish recovered by an electronic tag detector installed in the reduction plant at Isle aux Morts, Newfoundland, failed to reveal any wound infections or damage to internal organs caused by the presence of the tag in the body cavity (Winters 1975). Furthermore, healing of the incision was fairly rapid (≤6 mo). Thus, sustained mortality caused by the incision and the tag was probably not significant. However, one would expect that the impoundment and handling of the herring during the tagging process would result in a deterioration of condition (through scale loss, abrasions, etc.) causing initial mortality which should be reflected in a lower survival rate for those fish held for extended periods. Although it is impossible to determine the extent of these losses directly it is possible to obtain indirect estimates of such initial mortality. All the fish of each liberation were tagged from the same batch of herring held in the holding pounds or the bar-seine up to 2 or 3 days. The longer these fish were impounded the greater their scale loss and the poorer their physical condition. Consequently, I assume that any differences between returns from the 1st day's tagging of each liberation and returns from tagging on subsequent days (Table 1) are due to initial mortality result-

ing from effects of handling and impoundment and will reduce the effective number of fish tagged.

An estimate of this mortality (Ricker's Type A) can be obtained from a method described by Ricker (1958) and Dickie (1963). If two groups of tags from a common impounded group are released in subsequent days we may write the catch equation for the 1st day's releases and returns in logarithm form as follows:

$$\ln n_t = \ln PR + \ln N_t + \ln \frac{F}{Z}(1 - e^{-(Z)t})$$

$$= \ln PR + \ln N_t + \ln A$$

where $n_t$ = number of tagged fish returned during time $t$; $N_t$ = number of tagged fish present at the beginning of time $t$; F = instantaneous rate of fishing mortality during time $t$; Z = instantaneous rate of total mortality during time $t$; R = fraction of the tagged fish which survive or retain their tags up to the beginning of time $t = 0$; P = the fraction of total recaptures during time $t$ which are returned.

Similarly for the 2nd day's releases we may write:

$$\ln n_t' = \ln P'R' + \ln N_t' + \ln A$$

If sustained total mortality rates from these two groups are the same, then we obtain by subtraction:

$$\ln n_t - \ln n_t' = \ln PR - \ln P'R' + \ln N_t - \ln N_t' \quad (1)$$

Thus, the difference in the position of parallel lines drawn through logarithms of returns of two groups of tags reflects both difference in numbers initially released and differences in the magnitude of Type A errors (Dickie 1963). Consequently, if we assume PR = 1 we obtain from the above equations a value for $-\ln P^1R^1$ that is an estimate of the relative initial tagging mortality (instantaneous) for the 2nd day's releases.

The logarithms of annual returns for each day of a liberation series are plotted in Fig. 2 for the 1970 and 1971 tagging experiments. Straight lines have been fitted by eye for each tagged group. Therefore, total mortalities within releases of the same liberation series are the same and consequently the relative magnitude of Type A errors can be evaluated, using equation (1), by comparing differences in positions of lines between the different days' releases. The results (Table 2) reveal that initial tagging mortality ($-\ln P^1R^1$) does occur and that relative tagging survival rate ($P^1R^1$) decreases substantially with the duration of impoundment. For example, when the March 4, 1970 returns are corrected for the difference in the initial numbers tagged, tags from that day's

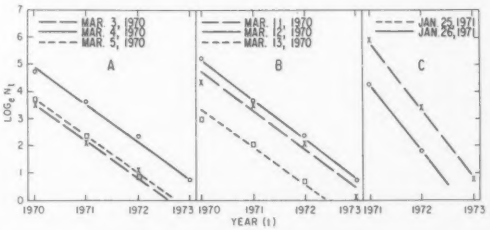


FIG. 2. Logarithms of annual recoveries from tag releases in Area J in 1970 (A, B) and 1971 (C).

releases were returned only 88% as commonly as the March 3, 1970 releases; similarly for the 1971 Bay de Loup experiment, tags from the 2nd day's releases were returned only 19% as commonly as the 1st day's releases. Estimates of relative tagging survival by the above method agree well with estimates derived from the ratio of percentage returns from subsequent days' releases compared with that of the initial day for each liberation series (Table 2, last column).

The estimates of relative tagging survival ($P^1R^1$) given in Table 2 have been used to calculate the maximum effective number of fish tagged by simple multiplication of $P^1R^1$ or the ratio of percentage returns with the number released during that day and accumulated for the liberation series (Table 3). The two estimates of effective number of fish tagged agree reasonably well and indicate that during the 1970 tagging experiment minimum estimates of initial tagging mortality ranged from 4 to 49% with an average for the entire experiment of 32% compared with 45.5% for the 1971 releases.

## Estimate of Tag Extrusion During Spawning

Examination of tagged herring recovered by a special tag detection unit (Winters 1975) suggested that tag loss during the spawning season (May–June for spring-spawners, August–September for fall-spawners) was substantial, particularly for those tags that had not become attached to internal organs. According to Ricker (1958) such a tag loss will produce a recapture curve which will be either concave upwards or best fitted by two straight lines. Tag returns from the 1970 and 1971 tagging experiments along southwest Newfoundland (prespawning spring and fall-spawners) and in the southern Gulf of St. Lawrence (ripe and running spring-spawners at Magdalen Islands, spent fall-spawners at Gaspé) have been separated by area and expressed in terms of tag recoveries per unit effort from effort data given in Winters and Hodder (1975). These have been transformed to natural logarithms and

TABLE 2. Calculation of the relative magnitude of Type A errors by the method of Ricker (1958) and Dickie (1963). The ratio of percentage tag returns is calculated from the data given in Table 1.

| Liberation series | Day released | $N_0$ | $\ln N_0$ | Calc. $\ln n_{1972}$ | $-\ln P^1R^1$ | $P^1R^1$ | Ratio of % returns |
|---|---|---|---|---|---|---|---|
| A1 | Mar. 3/70 | 1100 | 7.00 | 0.80 | | | |
| | Mar. 4/70 | 4600 | 8.43 | 2.10 | | | |
| | Difference | | −1.43 | −1.30 | 0.13 | 0.88 | 0.85 |
| A1 | Mar. 3/70 | 1100 | 7.00 | 0.80 | | | |
| | Mar. 5/70 | 2700 | 7.90 | 0.95 | | | |
| | Difference | | −0.90 | −0.15 | 0.75 | 0.47 | 0.45 |
| A3 | Mar. 11/70 | 2000 | 7.60 | 1.85 | | | |
| | Mar. 12/70 | 7000 | 8.85 | 2.25 | | | |
| | Difference | | −1.25 | −0.40 | 0.85 | 0.43 | 0.57 |
| A3 | Mar. 11/70 | 2000 | 7.60 | 1.85 | | | |
| | Mar. 13/70 | 2800 | 7.94 | 0.60 | | | |
| | Difference | | −0.34 | 1.25 | 1.59 | 0.20 | 0.18 |
| B1 | Jan. 25/71 | 4800 | 8.48 | 3.30 | | | |
| | Jan. 26/71 | 5200 | 8.56 | 1.70 | | | |
| | Difference | | −0.08 | 1.65 | 1.68 | 0.19 | 0.14 |

TABLE 3. Estimation of effective number of fish tagged for the 1970 (A) and 1971 (B) tagging experiment.

| Liberation series | Effective no. of fish tagged: Calc. from ratio % returns | Effective no. of fish tagged: Calc. from $P^1R^1$ | Avg. | Actual no. released | Estimated minimum initial mortality rate (%) |
|---|---|---|---|---|---|
| A1 | 6,250 | 6,400 | 6,350 | 8,400 | 24.4 |
| A2 | 4,600 | — | 4,600 | 4,800 | 4.2 |
| A3 | 6,500 | 5,600 | 6,050 | 11,800 | 48.7 |
| Total | | | 17,000 | 25,000 | 32.0 |
| B | 5,050 | 5,800 | 5,450 | 10,000 | 45.5 |

are plotted against time in Fig. 3. The tag recovery rates in southwest Newfoundland during March–April of 1970 and January–April of 1971, the only recovery periods in those experiments completely prior to spawning, are substantially greater than would be expected from the trend in the rates of tag recovery in periods subsequent to spawning. A straight line (A) has been fitted to these latter points and is joined by a line (B) from the point representing the prespawning recovery in Area J in 1970 and 1971. A line drawn parallel to line A from line B, i.e. line C, would thus represent the expected recovery rate if no tag loss were incurred during spawning. A comparison of the observed and expected recovery rates in this manner reveals that tag recoveries in 1972, for example, were only 4% of what they would have been in the absence of tag loss during spawning. Similar tag losses are evident from the trend of tag recoveries from the 1971 tagging experiment along southwest Newfoundland but not from the two tagging experiments in the southern Gulf of St. Lawrence in 1970. The apparent lack of tag loss during spawning in the Magdalen Islands releases is due to predominance of ripe and running fish (spring-spawners) in the herring used for tagging and thus tag loss during spawning would have already occurred before those fish became subject to exploitation during their feeding season in the American Bank area (Fig. 1) in July and August. The fish used in the Gaspé tagging experiment were predominantly postspawners (autumn-spawners) (J. S. Beckett, Department of Fisheries and the Environment, Fisheries and Marine Service, Ottawa, personal communication) and thus, loose tags would have a full year before the next spawning season to become attached to some internal structure. The lack of substantial tag loss indicated by the trend in recovery rates of tags released in the Gaspé

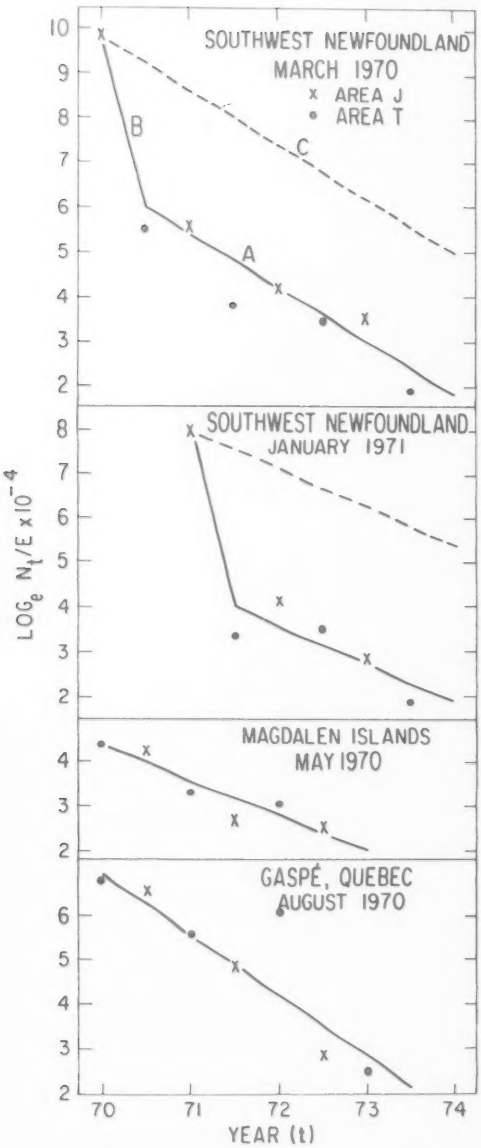


FIG. 3. Logarithms of annual tag recoveries per unit effort from tagging experiments conducted in Areas J and T in 1970–71.

experiment may therefore be explained on this basis.

A second estimate of tag extrusion during spawning may be obtained from a comparison of the effective number of marked fish alive before spawning and those marked fish containing tags which are alive after the completion of spawning, adjusting for interim fishing and natural mortality rates. The 1970 tagging along southwest Newfoundland fits the requirements since those fish were tagged just prior to spring spawning and substantial returns from this experiment were obtained in the 1971 winter fishery along southwest Newfoundland which began 1–2 mo after fall spawning had ended.

An estimate of the surviving tagged fish containing tags from the 1970 releases can be derived from a comparison of the exploitation rates (R/M) during the recovery period in southwest Newfoundland in 1970 and 1971, providing effort data are available to adjust the tag returns to an equivalent exploitation rate (assuming that the catchability coefficient remained constant). Effort data are available for these years (Winters and Hodder 1975) and the relevant data are summarized below (op. = operating):

| | 1970 | 1971 |
|---|---|---|
| No. tagged fish (M) | 17,000 | ? |
| Effective tag recapture (R) | 1,150 | 185 |
| Catch (t) (C) | 25,000 | 104,000 |
| Effort (op. days) (E) | 602 | 4,230 |

We may thus calculate the number of fish of the 1970 tagging experiment, surviving to the beginning of the 1971 winter fishery as follows:

$$\frac{E_{t+1} \cdot R_t}{E_t \cdot M_t} = \frac{R_{t+1}}{M_{t+1}} \qquad (2)$$

Whence,

$$M_{71} = \frac{R_{71} \cdot E_{70} \cdot M_{70}}{E_{71} \cdot R_{70}}$$

$$= \frac{185 \times 602 \times 17{,}000}{4{,}230 \times 1{,}150}$$

$$= 389$$

Thus, the number of tagged fish declined from 17,000 in March 1970 to 389 fish in November 1970 representing an instantaneous mortality rate (Z) of 3.78. The actual mortality rate of the stock over this period was approximately 0.60 (Winters and Hodder 1975) implying an apparent Z = 3.18 as the tag loss rate due to extrusion during spawning. The above estimate of 389 surviving fish therefore suggests that 96% of the internal tags from the 1970 tagging experiment along southwest Newfoundland were extruded and effectively removed from the marked population during spawning in the southern Gulf of St. Lawrence. This estimate is identical to that obtained above from a comparison of pre- and postspawning tag return rates per unit of effort.

## Discussion and Conclusions

Substantial initial tagging mortality resulting from deteriorating body condition due to effects of holding fish prior to tagging operations and tag application has been demonstrated for a variety of species including herring (Jensen 1955), North Sea sole (Kotthaus 1963), salmon (Haitt 1963), and haddock (McDermott and Livingstone 1963). For example, Jensen (1955) graded live herring into four categories according to degree of scale loss and compared subsequent returns of tagged fish from the four categories. The results indicated that the return rate from the category containing fish in the poorest condition was <10% of that of the best condition category. An increase in severity of scale loss related to duration of impoundment was also evident in tagging experiments carried out along southwest Newfoundland and the analyses of subsequent tag recaptures reinforce previous conclusions that maximum tagging efficiency will only be achieved in experiments involving a minimum of handling activity and impoundment time prior to release of the tagged fish. Thus, a fresh batch of live fish for tagging purposes should be obtained as frequently as possible. If this cannot be achieved and the tagging project is of such a nature as to allow recording of fish condition at release, such data could be of great value in evaluating and adjusting for such biases introduced into recapture data (Jensen 1955). For species, such as herring, that school in large numbers and are highly susceptible to handling mortality, rapid, mass taggings are desirable and are thus not conducive to recording of fish condition; however, data on the time of tag release relative to holding time (as in the tagging experiment described above) would allow estimation of initial tagging mortality.

An additional critical factor affecting the magnitude of long-term returns from herring with abdominally inserted tags is the proximity of the tagging period to the spawning season. Results obtained in this study suggest that a high proportion of internal tags (presumably loose in the body cavity or embedded in the gonads) applied shortly before spawning are extruded during the spawning act. This observation is supported by data on the location of the magnetic tags in the body cavity of herring recaptured by electronic tag detector after varying periods of release (Winters 1975). In fish 2 mo at large and prior to spawning, 96% of the internal tags were either loose in the body cavity or embedded in the gonads. After 7 mo at large and also prior to spawning, 80% of the tags were loose or embedded in the gonads, the remainder being attached to the body wall and mesentery. After 10 mo at large and in postspawning fish all tags were attached to the body wall, none being found embedded in the gonads or loose in the body cavity. Thus, the most appropriate time to conduct a tagging experiment involving abdominally inserted tags is shortly after spawning has been completed.

### Acknowledgments

Dr V. C. Anthony reviewed this paper and his many helpful suggestions are gratefully acknowledged.

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# Habitat Partitioning in a Freshwater Fish Community[1]

EARL E. WERNER, DONALD J. HALL, DENNIS R. LAUGHLIN,
DONALD J. WAGNER, LENI A. WILSMANN, AND FREDERICK C. FUNK

*W. K. Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, Michigan 49060 USA*

WERNER, E. E., D. J. HALL, D. R. LAUGHLIN, D. J. WAGNER, L. A. WILSMANN, AND F. C. FUNK. 1977. Habitat partitioning in a freshwater fish community. J. Fish. Res. Board Can. 34: 360–370.

The patterns of habitat utilization in the littoral zone fish community of two small southern Michigan lakes were examined. Abundance and habitat use of the fish were quantified by underwater transect censuses. The sunfishes (Centrarchidae) dominated the communities numerically and by weight, with two species, the bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) accounting for 85% of the community biomass. Spatial (habitat) segregation of species was evident along gradients of depth (distance from shore), vertical height in the water column, and vegetational structure. Several species showed intraspecific differences in the spatial distribution of size-classes. Comparative analyses of habitat use in the two lakes suggest that small size-classes are confined by predation to areas of dense cover and that within these areas competition determines space utilization by different species. The patterns of habitat use are discussed in relation to the food habits and morphology of species in this community. Only one clear case of segregation of two species by food size occurs (bass and bluegill); most other species segregate predominantly by habitat. The rarer centrarchids show strong niche complementarity with the codominant bass and bluegill.

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Les auteurs étudient les modalités d'utilisation de l'habitat par des communautés de poissons de la zone littorale de deux petits lacs du Michigan méridional. L'abondance des poissons et l'usage qu'ils font de l'habitat ont été quantifiés par recensements et observations sous-marins sur des coupes transversales. Les crapets (Centrarchidae) dominent les communautés en nombre et en poids. Deux espèces, le crapet arlequin (*Lepomis macrochirus*) et l'achigan à grande bouche (*Micropterus salmoides*), représentent 85% de la biomasse des communautés. La ségrégation spatiale (habitat) est évidente le long de gradients de profondeurs (distance du rivage), dans la hauteur verticale de la colonne d'eau et selon la structure de la végétation. Plusieurs espèces montrent des différences intraspécifiques dans la distribution spatiale des classes de longueur. Des analyses comparatives de l'utilisation de l'habitat dans les deux lacs donnent à croire que les petites classes de longueur sont confinées par la prédation aux régions de dense couverture et que, dans les limites de ces régions, la compétition détermine l'utilisation de l'espace par les différentes espèces. On analyse les modalités de l'utilisation de l'habitat en relation avec les mœurs alimentaires et la morphologie des espèces dans la communauté. Il ne se présente qu'un cas bien défini de ségrégation de deux espèces suivant la grosseur de la nourriture (achigan et crapet arlequin); la plupart des autres espèces se séparent en grande partie selon l'habitat. Les centrarchidés plus rares sont fortement complémentaires, quant à la niche, des achigans et des crapets arlequins co-dominants.

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STUDIES of resource partitioning among species in a community provide considerable insight into the nature of interspecific interactions and critical dimensions of the niche. The patterns in resource partitioning, however, are known only in a few groups of organisms, the majority of which are terrestrial (cf. Schoener 1974). Even in the better known communities the data are usually incomplete since measuring utilization on certain resource dimensions is considerably more difficult than others. For instance, birds are relatively easy to observe and this has resulted in many excellent studies of the habitat utilization of coexisting

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species. Relevant data on food use of birds, however, are limited (Hespenheide 1975). The converse holds for temperate zone freshwater fish where an extensive literature exists on food habits, but few studies have examined habitat utilization among coexisting species.

This study concerns the patterns of habitat utilization in a freshwater fish community. The community studied is typical of small (<100 ha) glacial lakes of central North America characterized by protected littoral zones with soft, weedy bottoms. The fish communities of these lakes are largely limited to the littoral zone and are often dominated by the freshwater sunfishes (Centrarchidae). The minnows (Cyprinidae) are of secondary importance, particularly in terms of total biomass. A number of other families are usually represented by one or several species in these communities (e.g. Percidae, Catostomidae, Cyprinidontidae, Esocidae, Amiidae, Atherinidae, and Ictaluridae).

## Study Sites and Methods

The littoral zone fish communities of two small southern Michigan lakes were investigated during late July and early August 1975. Lawrence Lake, 2.1 km east of Hickory Corners, Barry County, Michigan, is an oligotrophic, marl lake, 4.9 ha surface area and 12.6 m maximum depth. The littoral zone is composed of a nearly continuous marl bench extending as much as 20 m from shore to a depth of about 1.5 m, from which point the bottom slopes steeply to a depth of 6 or 7 m (Fig. 1). Further morphometric and limnological details may be found in the contributions referred to in Wetzel (1975). Three Lakes, about 13 km southwest of Lawrence Lake in Kalamazoo County, is more productive than Lawrence Lake and does not contain marl deposits; Three Lakes is about 10 ha in surface area and 10 m maximum depth. The configuration of the littoral zone is similar to that of Lawrence. Both lakes were thermally stratified with the metalimnion commencing at a depth of 5–6 m. This depth coincides with the deepest extent of rooted aquatic plants. The hypolimnia of both lakes remained aerobic through the summer.

The littoral zones were each divided into two morphometric regions for sampling: bench — gently sloping region 0–1.5 m deep and slope — steeply sloping region 1.5–6 m deep. This division also coincides with important vegetation changes. In Lawrence Lake the bench is characterized by patches of three vegetation types that are continuous from the shore to about the 1.5 m contour and extend along the shore for 20–100 m. These are 1) exposed marl sediments with sparse cover (usually <10%) of very short *Chara* and *Utricularia*, 2) dense stands (usually >80% cover) of *Scirpus subterminalis*, *Najas flexilis*, and *Chara* roughly 10–15 cm tall and, 3) dense stands of water lilies (*Nuphar* sp and *Nymphaea* sp) with

FIG. 1. Morphometric map of Lawrence Lake. Heavy dashed lines locate the bench transects and the solid lines locate the slope transects.

an understory of *Scirpus*, *Najas*, or *Chara* (see Rich et al. 1971). The slope of Lawrence Lake is covered almost exclusively with a dense stand of *S. subterminalis* about 0.5 m tall. In Three Lakes patches of vegetation similar to (2) and (3) of Lawrence Lake cover the bench. The vegetation consists almost exclusively of dense stands of *Chara*, some with an overstory of water lilies. The slope of Three Lakes is covered with numerous patches of tall (1–1.5 m) *Potamogeton* spp., *Myriophyllum* sp., *Chara*, and *Utricularia*, forming a rather open canopy interspersed with areas of bare organic sediments.

The bench and slope regions were censused independently. On the bench, a team of three observers swam transects parallel to the shore employing mask and snorkel (Fig. 2). The slope region was censused in a similar fashion by a team of five observers using mask and snorkel or SCUBA gear (Fig. 2). Observers close to the bottom counted all fish within 1 m of the bottom; fish in the remainder of the water column were counted by the observer over-

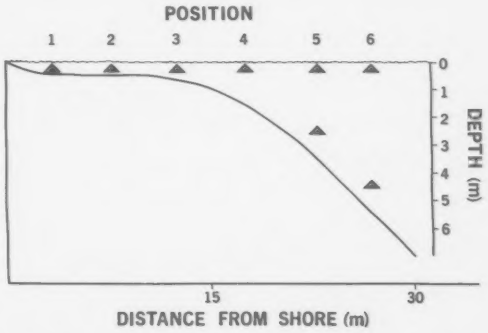


FIG. 2. Schematic profile of the littoral zone of Lawrence Lake. Triangles represent positions of underwater observers. The bench transects included positions 1–3 and the slope transects positions 4–6.

head. This division of the water column permitted more accurate counts and enabled us to distinguish fish associated with the bottom from those found in the water column.

Each fish encountered was identified to species (except for three cyprinids) and placed in one of three size-classes (small <7.5 cm, medium 7.5–12.5 cm, large >12.5 cm). Fry (<2 cm) were not counted. The data were recorded on clear acetate sheets with a wax pencil. Length was estimated from reference marks on the acetate sheets. Habitat (vegetation) association and vertical position in the water column were noted. Visibility was adequate for observers to see one another, permitting synchronous team movements. Counting duplication was minimized by prearranged limits to the sample field of each observer or, when uncertainty arose, by hand signals. On five occasions an additional diver was stationed either ahead, outside, or behind the diving team to observe the behavior of the fish. The fish filtered back through the counting team and did not introduce significant error by swimming ahead of the observers or circling out around them. Some fish, of course, were obscured by vegetation but they were predominantly fry that were not censused. With good visibility, underwater counts are in general more accurate than other nondestructive sampling techniques (cf. Northcote and Wilkie 1963).

Transect lengths ranged from 100 to 330 m. In Lawrence Lake nearly the entire littoral region was censused (see Fig. 1). In Three Lakes only one-half of the littoral zone was censused owing to the larger size of the lake and some areas of dredging and beach activity. Transects were censused during midday (1300–1600) and replicated 2–4 times on different dates. Two complete censuses of Lawrence Lake are presented in the Appendix. Replicate counts and the distribution of fish are very similar on the different dates. Lawrence Lake was also censused once at dusk (2000–2100) in an attempt to discern any diel differences in fish distributions.

Numerous transects were also swum across the open water portions of the lakes. The only fish ever encountered in the offshore surface waters were centrarchid fry. Fish were never seen in deep water (>6 m) where temperatures were <14°C. For this reason we limited our attention to the littoral zone community.

## Results

### Species Composition and Relative Abundance

The littoral zone fish communities of Lawrence Lake and Three Lakes are similar in species composition (Table 1) and are typical of the small glacial lakes in southern Michigan. Fifteen species were regularly censused in Lawrence Lake and 16 in Three Lakes; 14 of these species are common to both lakes. These totals do not include a few (less than five) species that are difficult to identify underwater (e.g. darters) or rarely encountered because of their location (*Fundulus*, *Ictalurus*). Both communities are rich in centrarchids (seven species regularly censused in Lawrence Lake, six in Three Lakes); the same species occur in both lakes except for the longear sunfish (*Lepomis megalotis*), which is absent in Three Lakes (Table 1). The cyprinids are represented by five species in Three Lakes and four in Lawrence Lake. The remaining families are represented by only one or a few species.

The absolute and relative abundances of the more numerous species are presented in Table 2. The centrarchids constitute 70% of the fish in the littoral zone of Lawrence and 60% in Three Lakes primarily due to the abundance of the bluegill (*L. macrochirus*). Each centrarchid is more abundant in Three Lakes than Lawrence Lake with the notable exception of the bluegill. The only other species that constitute more than 4% of the individuals in these communities are the perch in Lawrence Lake and the combined species of the shiner complex.

To provide another measure of the importance of each species, the abundance estimates were converted to biomass values using mean weight estimates of each species from Brown and Ball (1942) and other sources (cf. Table 1). The biomass estimates demonstrate even more strikingly the dominance of the centrarchids, which comprise 92 and 94% of the biomass in Lawrence Lake and Three Lakes, respectively (Table 3). The bluegill (68 and 60%) and largemouth (*Micropterus salmoides*) bass (18 and 25%) are the dominant species, which is a typical pattern for small southern Michigan lakes (e.g. Brown and Ball 1942). No other species contributes more than 6% of the community biomass.

### Habitat Utilization in Lawrence Lake

The distribution of centrarchids in relation to lake morphometry (Fig. 3, see also the Appendix) reveals considerable separation among these species according to the depth of the water column (thus, also distance from shore). The green (*L. cyanellus*) and longear sunfishes are largely restricted to the shallow bench region, while the bluegill and black crappie (*Pomoxis nigromaculatus*) are found predominantly in the deeper littoral. The bass and pumpkinseed (*L. gibbosus*) are more uniformly distributed across depths, with the bass more abundant in the deeper water and the pumpkinseed in the shallower water.

The centrarchids also segregate on the vertical dimension, utilizing different strata in the water column. Virtually all (99%) of the pumpkinseeds and longears were encountered close to the

TABLE 1. Species composition of Lawrence Lake and Three Lakes. The size ranges are average size during the second growing season (1 annulus) to the upper limit of the normal adult range. Data are from statistics of the Department of Natural Resources, State of Michigan, where available, and Trautman (1957) or Carlander (1969) otherwise.

| Family/Species | Lawrence Lake | Three Lakes | Size range: Total length (*cm*) | Size range: W (*g*) |
|---|---|---|---|---|
| Centrarchidae | | | | |
| *Micropterus salmoides*, largemouth bass | + | + | 15.5– 50.3 | 48 –1900 |
| *Pomoxis nigromaculatus*, black crappie | + | + | 12.9– 42.7 | 31 – 476 |
| *Lepomis macrochirus*, bluegill | + | + | 8.6– 23.1 | 11.3– 250 |
| *L. gibbosus*, pumpkinseed | + | + | 8.4– 19.8 | 11.3– 184 |
| *L. gulosus*, warmouth | + | + | 7.9– 19.1 | 10 – 164 |
| *L. cyanellus*, green sunfish | + | + | 7.6– 14.5 | 8 – 68 |
| *L. megalotis*, longear sunfish | + | 0 | 6.4– 10.9 | 5.4– 25 |
| *Ambloplites rupestris*, rock bass | + | 0 | 8.0– 25.0 | 9 – 350 |
| Percidae[a] | | | | |
| *Perca flavescens*, yellow perch | + | + | 11.7– 31.2 | 17 – 380 |
| Cyprinidae | | | | |
| *Notemigonus crysoleucas*, golden shiner | + | + | 8.1– 17.8 | 3 – 66 |
| *Notropis cornutus*, common shiner | + | + | 5.1– 17.8 | 0.7– 50 |
| *N. heterodon*, blackchin shiner | + | + | 3.5– 6.4 | ~1 |
| *N. heterolepis*, blacknose shiner | + | + | 4.6– 6.4 | ~1 |
| *N. anogenus*, pugnose shiner | 0 | + | 3.3– 4.8 | ~1 |
| Catostomidae[b] | | | | |
| *Erimyzon sucetta*, lake chubsucker | + | + | 8.4– 25.4 | ? – 230 |
| Esocidae | | | | |
| *Esox lucius*, northern pike | + | + | 39.6–100.6 | 357 –6650 |
| *E. americanus vermiculatus*, grass pickerel | + | + | 19.8– 27.7 | 40 – 116 |
| Amiidae | | | | |
| *Amia calva*, bowfin | + | + | 23 – 63.5 | ? –2500 |
| Atherinidae | | | | |
| *Labidesthes sicculus*, brook silverside | 0 | + | 5 – 9.6 | ~1.3 |
| Cyprinodontidae | | | | |
| *Fundulus diaphanus*, banded killifish | 0 | + | 3.8– 8 | ~1 |
| *F. notatus*, blackstripe topminnow | 0 | + | 3.8– 7.6 | ~1 |
| Ictaluridae[c] | | | | |
| Salmonidae[d] | | | | |

[a]Darters are known to be present but were not identified to species.

[b]An occasional sucker (other than the lake chubsucker) was encountered in Lawrence Lake but not identified to species.

[c]Bullheads were noted in Three Lakes but were not identified to species.

[d]Both lakes have been stocked at times with *Salmo gairdnerii*; these have been sighted only occasionally in Three Lakes.

bottom (within 0.5 m). The bass, bluegill, and crappie were found up in the water column.

The bluegill and bass further exhibited marked intraspecific size-class differences in vertical distribution. The bluegill population was sampled specifically to obtain a finer resolution of these differences. The water column was divided into three strata: 1) 0–0.5 m above the sediments, i.e. to the top of the vegetation, 2) 0.5–1 m above the bottom, and 3) the remainder of the water column. These observations were performed on one date at positions 4–6 (Fig. 4). The majority of small bluegills were confined to the 0.5–1-m stratum just above the vegetation. The larger size-classes were found higher in the water column. The size-classes of the bass were distributed in a similar fashion. When centrarchid fry were encountered in the littoral zone they were found in the vegetation.

The perch (Percidae) was broadly distributed

TABLE 2. Absolute (number per 100 m of littoral zone) and relative abundances of species in Lawrence and Three Lakes. Since observers were positioned such that the entire littoral zone was censused (from shoreline to deepest occurrence of macrophytes), the data are the sums of the average number of individuals per 100 m of transect across all positions.

| Lawrence Lake | | | Three Lakes | | |
|---|---|---|---|---|---|
| Species | Abundance | % | Species | Abundance | % |
| Bluegill | 411.2 | 56.87 | Bluegill | 326.0 | 40.68 |
| Shiner complex[a] | 189.9 | 26.26 | Shiner complex | 288.1 | 35.90 |
| Bass | 53.5 | 7.39 | Bass | 65.5 | 8.10 |
| Perch | 31.0 | 4.28 | Pumpkinseed | 51.9 | 6.47 |
| Crappie | 17.1 | 2.36 | Crappie | 19.4 | 2.42 |
| Pumpkinseed | 12.4 | 1.71 | Silverside | 10.9 | 1.36 |
| Longear | 5.7 | 0.78 | Golden shiner | 9.9 | 1.23 |
| Green | 1.0 | 0.13 | Perch | 9.8 | 1.22 |
| Pike | 0.6 | 0.08 | Common shiner | 7.8 | 0.97 |
| Bowfin | 0.5 | 0.07 | Warmouth | 4.2 | 0.52 |
| Warmouth | 0.1 | 0.01 | Chubsucker | 3.9 | 0.48 |
| | | | Green | 3.0 | 0.37 |
| | | | Bowfin | 0.4 | 0.04 |
| | | | Pike | 0.3 | 0.03 |
| Total fish/100 m | 723.05 | | | 801.2 | |

[a]Composed of the blackchin, blacknose, and pugnose shiners. These species are very small and occur in mixed-species schools. They are virtually impossible to distinguish underwater so were counted as an inclusive "shiner complex".

TABLE 3. Percent contribution of each species to total community biomass. Numerical abundances were converted to biomass using estimates of mean individual weight primarily from Brown and Ball (1942).

| Lawrence Lake | | Three Lakes | |
|---|---|---|---|
| Species | % | Species | % |
| Bluegill | 68 | Bluegill | 60 |
| Bass | 18 | Bass | 24.5 |
| Perch | 5.1 | Crappie | 5.9 |
| Crappie | 4.7 | Pumpkinseed | 3.7 |
| Bowfin | 1.4 | Perch | 1.8 |
| Pike | 1.1 | Bowfin | 1.2 |
| Pumpkinseed | 0.8 | Shiner complex | 0.9 |
| Shiner complex | 0.5 | Pike | 0.6 |
| Longear | 0.4 | Golden shiner | 0.4 |
| Green | 0.06 | Common shiner | 0.4 |
| Warmouth | 0.006 | Warmouth | 0.3 |
| | | Green | 0.2 |
| | | Silverside | 0.06 |
| | | Chubsucker | 0.09 |

across depths (Fig. 3) and was always closely associated with the bottom. The shiner complex (Cyprinidae) was found in shallow water associated with dense *Chara* and *Scirpus* beds. No other species were abundant enough to permit good estimates of habitat use in Lawrence Lake.

Habitat utilization by these species is also influenced by vegetation structure. The proportion of each species found in the three vegetation types on the bench suggests that species segregating by depth also tend to utilize different vegetation types when co-occurring in shallow water (Fig. 5). Thus, the crappie, bluegill, and bass were more abundant among the water lilies, while the green and longear were more abundant over open sediments and the *Chara–Scirpus*. The perch and pumpkinseed which were more evenly distributed across depths were somewhat more evenly distributed among vegetation types. The vegetation on the slope was quite uniform; therefore, we did not attempt to discern any differences in species distributions related to vegetation in this region.

## DIEL MOVEMENTS IN LAWRENCE LAKE

Fish often exhibit diel movements in association with feeding which may permit ecological segregation of species that otherwise do similar things. Most species in this community are visual feeders, and the literature on their natural history indicates that movements are keyed primarily to dawn–dusk periods.

To determine if such movements could be detected in Lawrence Lake, fish were censused immediately after sunset (2000–2100) when light intensities (lux) were from two to four orders of magnitude lower than daytime values (cf. Haney

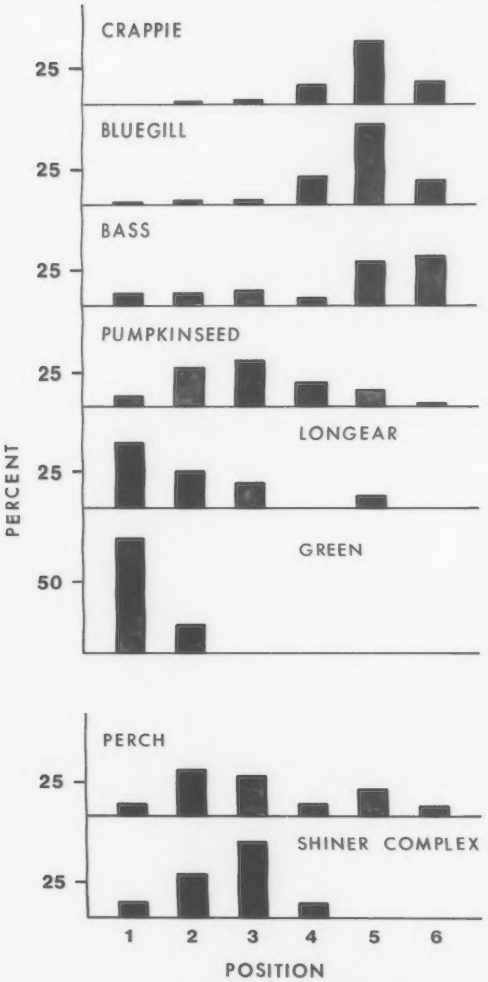


FIG. 3. Average proportional abundance of the eight most common species at midday in Lawrence Lake at each depth position (cf. Fig. 2). The centrarchids are grouped in the upper panel.

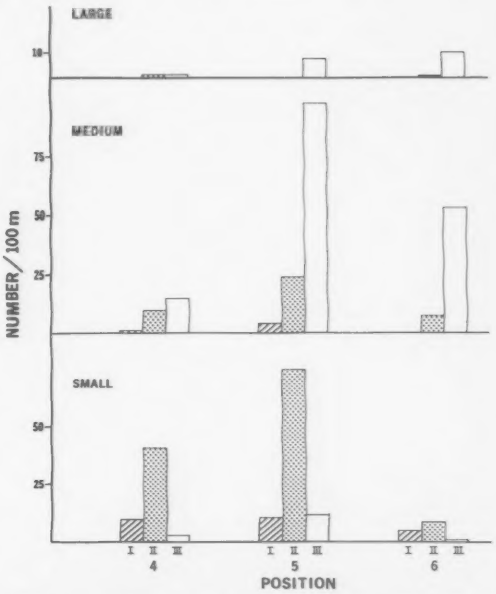


FIG. 4. Abundance of large, medium, and small bluegills on the slope of Lawrence Lake (positions 4–6) according to vertical location in the water column. I (cross-hatched histograms) is <0.5 m above the bottom (i.e. to the top of the vegetation); II (stippled histograms) is 0.5–1 m above the bottom; and III (clear histograms) is >1 m above the bottom.

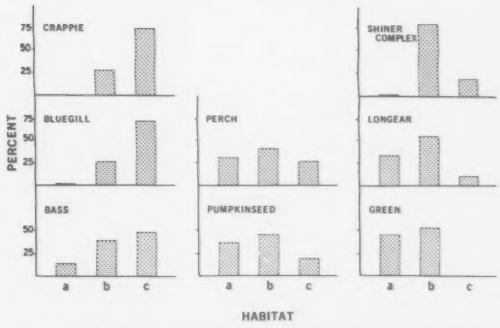


FIG. 5. Average proportional abundance of each species in the three different vegetation types on the bench (position 1–3) of Lawrence Lake. Type a is open marl bottom; type b is dense (>80% cover) *Chara-Scirpus*; and type c is lily pads.

and Hall 1975). Although the lake was censused only once at this time, the total number of individuals counted on the evening transects for most species were similar to the average of the replicated afternoon counts (e.g. bluegill, 380 and 411/100 m, respectively; bass, 55.7 and 53.5; crappie, 15.0 and 17.1).

The bluegill exhibits an inshore movement at dusk. In the evening the small size-class moved up the slope with a maximum abundance at the edge of the bench zone, i.e. position 4 (Fig. 6). The larger size-classes also moved toward the bench and higher up into the water column (Fig. 6). This inshore movement of all size-classes agrees with the observations of Baumann and Kitchell (1974). The small bass also moved inshore in the evening (Fig. 6). The distribution of larger bass showed little change. The crappies appear to move off the bench and higher up into

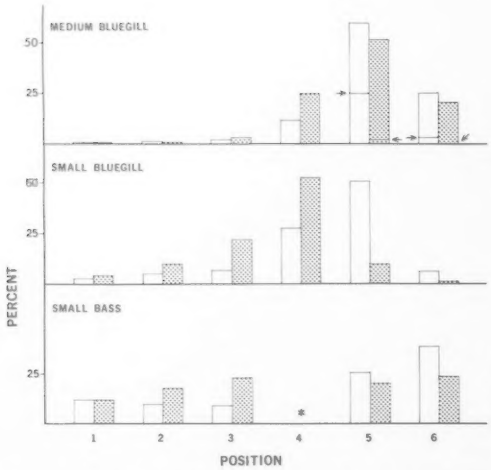


FIG. 6. Proportional abundance of several size-classes of the bluegill and bass at each depth position in Lawrence Lake. Clear histograms represent the midday distributions and stippled histograms the dusk distributions. Arrows separate the histograms of the medium bluegills into proportions encountered >1 m from the bottom (above) and <1 m from the bottom (below). The small bass at position 4 were not censused.

the water column at dusk. These observations, although tentative because of a single evening census, tend to support the findings of other investigators (Baumann and Kitchell 1974, Keast 1968) employing other techniques.

## HABITAT UTILIZATION IN THREE LAKES

Although the communities of Three Lakes and Lawrence Lake were similar in the habitat utilization by various species (Fig. 3 and 7), several species showed differences between communities suggesting the nature of species interactions. Additionally, the data from Three Lakes permitted quantification of the habitat distributions of some of the rarer species which was not possible in Lawrence Lake (e.g. the warmouth, *L. gulosus*, and lake chubsucker, *Erimyzon sucetta*, which were associated with the bottom and the common, *Notropis cornutus*, and golden, *Notemigonus crysoleucas*, shiners found up in the water column — Fig. 7).

An important difference is seen in the bass and bluegill which were more uniformly distributed across depths in Three Lakes than in Lawrence Lake (cf. Fig. 3 and 7). This difference is due almost entirely to a marked inshore shift in the distribution of the small size-classes of both species (Fig. 8); the medium and large size-

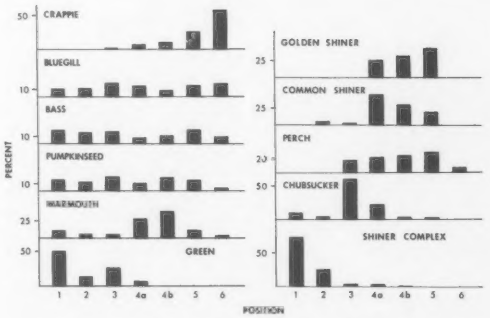


FIG. 7. Average proportional abundance of the eleven most common species at midday in Three Lakes at each depth position. The centrarchids are grouped in the left hand panel. An additional observer was employed at position 4 due to the increased width of the littoral zone in Three Lakes. Positions 1–3 and 5–6 correspond to the same depths as in Lawrence Lake. Position 4a was at 1.5 m and 4b at 1.7 m.

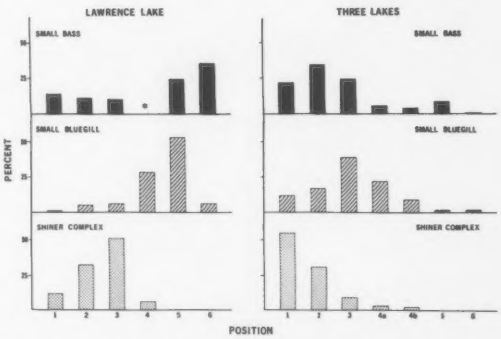


FIG. 8. Average proportional abundance of small bluegills, small bass, and the shiner complex according to depth position in Lawrence and Three Lakes. Note the compensatory habitat shift towards shore by the shiners in Three Lakes when all species are most abundant in shallower water. The small bass at position 4 in Lawrence Lake were not censused.

classes were distributed in much the same manner as in Lawrence Lake. The shift in the small size-classes appears to be associated with differences in the distribution of vegetation life forms in the two lakes. In Lawrence Lake the small size-classes were largely confined to the 0.5–1 m stratum above the dense *Scirpus* at positions 4–6 (Fig. 4). In Three Lakes the vegetation at positions 4b–6 formed a more open canopy; *Chara* on the bench was the only available dense cover. The shift in distribution of these small centrarchids was accompanied by a corresponding shift in the habitat

utilization of the shiner complex; in particular the separation between the small bluegills and the shiner complex was similar in the two lakes (Fig. 8).

## Discussion

The dominant bluegill and largemouth bass are fairly general in their habitat use and exhibit similar spatial distributions (especially the larger size-classes, cf. Fig. 3). Consideration of body size, food, and morphology indicates that these species are segregating on the food-size dimension of the niche (Werner 1977). The bluegill feeds on a general array of small-bodied invertebrates (Keast 1970; Seaburg and Moyle 1964; Gerking 1962, 1964), while the bass is almost totally piscivorous by the time it is 8–10 cm long (Keast 1970; Clady 1974; Kramer and Smith 1962).

The functional morphologies of the bluegill and bass are quite different. The bluegill has a small, protrusible mouth and a short, laterally compressed body with large mobile fins. The bass has the largest mouth of all the centrarchids and is more fusiform with smaller relative fin area. The differences in efficiency of these species when feeding on the spectrum of food sizes available has been quantified by Werner (1977) permitting calculation of the separation between the bass and bluegill on the food size axis. This separation closely approximates the "limiting similarity" predicted by current species packing theory (May and MacArthur 1972). In addition, the average weight of the bass is roughly twice that of the bluegill in accord with the differences in body mass among species of other groups segregating by food size. The bass and bluegill monopolize a broad range of the food sizes available in the littoral zone (Werner 1977) suggesting that to coexist, the remaining centrarchids should exhibit strong niche complementarity (Schoener 1974) with these two species.

Green sunfish and warmouth are intermediate between the bass and bluegill in their functional morphology and the food sizes utilized (e.g. Huish 1946; Larimore 1957; Etnier 1971; Werner and Hall 1976). The green sunfish shows strong niche complementarity with the bass and bluegill due to separation by habitat (Fig. 3, 7). The green is found in very shallow water next to the shore and the bass and bluegills in deeper water. Less is known about the warmouth. Its diet does not appear to differ much from that of the bass (Larimore 1957) and ecological segregation from the bass and bluegill apparently occurs by habitat and time of activity. We usually found the warmouth in dense vegetation near the bottom. Also, a study of the feeding chronology by Larimore (1957) suggests considerable nocturnal feeding. The green and warmouth are clearly segregated from each other by depth (Fig. 7).

The crappie also shows niche complementarity with the bass and bluegill. The crappie has a moderately large mouth and fine gill rakers, feeds on a broad range of prey sizes, and is more nocturnal than the bass and bluegill (Childers and Shoemaker 1953; Seaburg and Moyle 1964; Pearse 1919). Food studies indicate that it feeds primarily in the offshore water column, particularly on insects and Cladocera that migrate toward the surface at dusk and small fish (Seaburg and Moyle 1964; Keast 1968). Our direct observations also suggest an offshore movement of the crappie in the evening. Thus, the food of the crappie may be obtained in a different habitat than that of the bluegill and bass even though the midday distributions of all three are similar.

Observations of the two remaining centrarchids, the pumpkinseed and longear sunfish, reveal that they methodically search the bottom, characteristically either sucking in mouthfuls of sediments and spitting everything out in a cloud from which the prey is selected, or separating the prey in the buccal cavity and exhausting the sediments from the mouth and opercular openings. Both possess a sparse number of short gill rakers which presumably do not foul with sediments. Thus, these two species are segregated to a considerable extent from the other centrarchids due to their use of the sediments (Seaburg and Moyle 1964; Etnier 1971; Werner and Hall 1976; Sadzikowski and Wallace 1976; Minkley 1963; Mullan and Applegate 1970). They are separated from each other by depth in Lawrence Lake where the two coexist (Fig. 3).

In addition, the pumpkinseed is the only centrarchid in this community equipped with heavy, molariform, pharyngeal teeth, enabling it to crush the shells of molluscs, particularly gastropods, which may constitute up to 90% of its diet (Seaburg and Moyle 1964). This specialization is probably important in reducing competition with species such as the bluegill whose diet shows considerable overlap with that of the pumpkinseed. Seaburg and Moyle (1964) have shown, however, that this overlap declined late in the summer when food resources are lowest because the pumpkinseed switches to gastropods.

In general, the qualitative patterns of resource partitioning among the centrarchids are reasonably clear. In contrast, the information on the other species in these communities is not as complete. The food of the yellow perch (*Perca*

*flavescens*) changes with increase in body size from zooplankton to benthos and eventually to fish at lengths >140 mm (Moffett and Hunt 1945, Clady 1974, Keast 1970). It appears, however, to be a very opportunistic feeder (Scott 1955; D. J. Hall personal observations), readily switching to abundant prey forms and, in contrast with the centrarchids, is a very active and wide-ranging searcher. More detailed studies are required to interpret the importance of these characteristics in permitting coexistence with the centrarchids.

The minnows (Cyprinidae) show patterns of segregation and morphological differences that are similar to those noted among the centrarchids, emphasizing the common constraints the environment places on niche partitioning. The blackchin (*Notropis heterodon*) and blacknose (*N. heterolepis*) shiners occur in mixed species schools, but appear to partition the water column: the blackchin, with a terminal mouth and fine gill rakers, feeds on zooplankton and surface insects (Keast 1970), whereas the blacknose, with a ventroterminal mouth and coarse gill rakers (Ransbottom 1951), feeds more on weed surfaces and possibly the bottom. Two larger species, the golden and common shiners, are about the same size and often occur together in deeper water. The golden shiner schools in weedy areas of the littoral during the day and moves offshore at night to feed on zooplankton (Suffern 1973; Keast and Webb 1966). Little is known about the common shiner in lakes. Based on qualitative observations, ecological segregation between these larger shiners appears to parallel that between the crappie and bluegill.

Since the cyprinids are relatively small and specialized, they will most likely interact with specific size-classes of the centrarchids, which often exhibit dramatic changes in food habits with increases in size (e.g. Keast 1970). Thus, the strength of the interactions between given species will depend on the life-history stage. As an example in Fig. 8 we presented the distributions of the small size-classes of the bluegill and bass, and the shiner complex in Lawrence Lake and Three Lakes. All of these fish are found just above a very dense vegetation cover. Since fish of this size are most vulnerable to predation, we suggest that these distributions reflect the higher probability of predation in the more open areas (cf. Jackson 1961). The concurrent shifts inshore of the shiners and small bluegills in Three Lakes further suggest that competition is occurring among these small fish. Predation pressure may then confine small fish to particular habitat types, while competition apparently determines the spatial relations among species within these habitats. Absolute amounts and distribution of various habitat types may therefore profoundly affect recruitment into later life-history stages.

Our considerations of resource partitioning pertain to late summer when resource levels are known to be lowest in temperate zone lakes (Seaburg and Moyle 1964). Several studies indicate that overlap in the diet of coexisting fish is highest during the spring flush of food and declines as resource levels drop (Seaburg and Moyle 1964; Nilsson 1960). A similar pattern has been reported in the tropics (Zaret and Rand 1971). We have suggested (Werner and Hall 1976) that seasonal changes in diet overlap may be due in part to shifts in habitat use (cf. the compression hypothesis, MacArthur 1972). If this is the case, the relative flexibility of different species in their habitat use and ability to capitalize on seasonally abundant resources in different habitats may be an important determinant of species abundance in these communities. The bluegill in particular is exceptionally flexible (Keast 1970) and this may account for its dominance. The bluegill and perch are the only species which show a noticeable decline in abundance in Three Lakes (the more productive of the two lakes studied — see Table 2) and both have been singled out as being generalists and quite plastic in their resource utilization. This suggests that as resource levels increase above thresholds required by the more specialized species, generalists may decline owing to diffuse competition from the suite of specialists.

In summary, ecological segregation among species in these communities is primarily by habitat. This segregation occurs in relation to depth or distance from shore, vertical layer in the water column (including the sediment–water column distinction), and structural characteristics of the vegetation. Although there is only one clear cut case of two species (bass and bluegill) utilizing virtually the same array of habitats and segregating on the food size dimension, these are the two dominant species in the community. Other dimensions involved in segregation are time of day and food characteristics other than size. In a literature review, Schoener (1974) has shown that separation by habitat is the most frequent means of providing ecological segregation in terrestrial communities. Although relatively little information is available for aquatic systems, our data indicate that the patterns of ecological segregation in fish communities appear to be quite parallel to those found in other communities.

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## Appendix

The following data are the actual species counts from Lawrence Lake on July 22 (upper row of following table) and July 25 (lower row) when all transects were censused.

The data for the eight most common species are presented to illustrate the variability in the counts. The data are the total number of fish of each species counted at the specified position with all transects at that position combined. The combined transect lengths for all positions range from 575 to 605 m.

Various individual transects (cf. Fig. 1) were also replicated on other dates and the data used in the compilations in the text and the figures but omitted here.

| | Position | | | | | | |
|---|---|---|---|---|---|---|---|
| Species | 1 | 2 | 3 | 4 | 5 | 6 | Total |
| Bluegill | 24 | 61 | 98 | 462 | 1173 | 479 | 2297 |
| | 34 | 67 | 104 | 375 | 1399 | 367 | 2346 |
| Shiner complex | 112 | 415 | 652 | 153 | | | 1332 |
| | 140 | 265 | 550 | 58 | | | 1013 |
| Bass | 33 | 21 | 33 | 16[a] | 95 | 125 | 323 |
| | 24 | 36 | 38 | 18[a] | 108 | 98 | 322 |
| Perch | 11 | 55 | 45 | 16 | 36 | 17 | 180 |
| | 14 | 63 | 53 | 14 | 22 | 6 | 172 |
| Pumpkinseed | 7 | 24 | 33 | 19 | 11 | 1 | 95 |
| | 4 | 17 | 15 | 9 | 7 | 1 | 53 |
| Crappie | | | 4 | 10 | 14 | 23 | 51 |
| | | 1 | 2 | 14 | 34 | 39 | 90 |
| Longear | 17 | 10 | 2 | | 2 | | 31 |
| | 21 | 8 | 11 | | | | 40 |
| Green | 3 | | | | | | 3 |
| | 6 | 2 | | | | | 8 |

[a]Small bass were not counted at this position.

# Validity of the Subspecies Designation for the Dwarf White Sucker (*Catostomus commersoni utawana*)

R. J. BEAMISH

*Department of the Environment, Fisheries and Marine Service, Pacific Biological Station, Nanaimo, B.C. V9R 5K6*

AND E. J. CROSSMAN

*Department of Ichthyology and Herpetology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ont. M5S 2C6*

BEAMISH, R. J., AND E. J. CROSSMAN. 1977. Validity of the subspecies designation for the dwarf white sucker (*Catostomus commersoni utawana*). J. Fish. Res. Board Can. 34: 371–378.

A reexamination of white sucker populations originally used to document the subspecies validity and biology of a dwarf white sucker, *Catostomus commersoni utawana*, provided no evidence of two separate populations of different-sized individuals. Small mature white suckers and larger or normal-sized white suckers still were present, but there was a continuous range of sizes after maturity. After maturity males grew slower than females and had a higher rate of annual mortality. If the size separation originally used to separate the two forms was applied to this study, then all normal-sized white suckers in this study were females and would have had to reproduce with smaller males. Also, no meristic or morphological evidence for justification of a subspecies designation for the dwarf form of white sucker in this lake was found. Because it was also known that considerable variation in the size of white suckers at maturity occurs throughout the range of this species, it was concluded that the existence of a distinct dwarf subspecies has not yet been demonstrated.

BEAMISH, R. J., AND E. J. CROSSMAN. 1977. Validity of the subspecies designation for the dwarf white sucker (*Catostomus commersoni utawana*). J. Fish. Res. Board Can. 34: 371–378.

Un réexamen des populations de meuniers noirs utilisées au début pour démontrer la validité d'une sous-espèce et la biologie d'un meunier noir nain, *Catostomus commersoni utawana*, n'indique pas qu'il s'agisse de deux populations séparées d'individus de tailles différentes. De petits meuniers noirs mûrs et des meuniers noirs plus grands ou de taille normale sont encore présents, mais il y a cependant une gamme continue de tailles après la maturité. Après la maturité les mâles croissent à un rythme plus lent que les femelles et ont un taux plus élevé de mortalité annuelle. Si l'on applique à cette étude la longueur originellement utilisée pour séparer les deux formes, tous les meuniers noirs de taille normale dans la présente étude sont des femelles, et il aurait fallu qu'elles se reproduisent avec des mâles plus petits. En outre, nous n'avons pas trouvé de caractères numériques ou morphologiques pour justifier le statut de sous-espèce à la forme naine du meunier noir dans ce lac. Etant donné de plus qu'il y a variation considérable dans la taille des meuniers noirs à la maturité dans toute l'aire de répartition de l'espèce, nous concluons que l'existence d'une sous-espèce naine distincte n'a pas encore été démontrée.

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IN 1890 Mather described a new species of white sucker from the Blue Mountain Lake region in northern New York state as "the June sucker" *Catostomus utawana*. Mather considered that this species was distinct from the white sucker, *Catostomus commersoni*, because of its small size at maturity and its later spawning season. In 1937 Dence, working in the same area as Mather, observed that "there are two kinds of common suckers in Wolf Pond [Wolf Lake]. During early June many dwarf suckers (*Catostomus commersonii utawana*) ranging [in standard length] from about 5 to 8 inches in length were spawning in the two principal inlet streams only a short distance from the pond. Larger suckers (*Catostomus commersonii commersonii*) ranging from 12 to 18 inches in length, were taken during this same period at various places in the pond itself". A comprehensive account of the

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biology of the dwarf sucker was published by Dence (1948) based on a study of white suckers from Wolf Lake, situated less than 40 km from the location of Mather's original samples. Dence believed two forms existed sympatrically in Wolf Lake, but he considered there was insufficient taxonomic separation to merit a species distinction and adopted the subspecies designation *Catostomus commersoni utawana* Mather, that had previously been suggested by Greeley and Green (1931). The existence of a small form of white sucker has since been recognized by other workers (Kendall and Dence 1929; Hubbs and Lagler 1964; McPhail and Lindsey 1970) and the occurrence of small-sized mature white suckers is not an uncommon observation throughout the range of the white sucker.

The recognition of subspecies within a small lake poses some interesting questions concerning the mechanism of isolation of the forms because subspecies are normally allopatric and interbreed where they come in contact. Before consideration could be given to the origin of dwarf forms of white suckers it was necessary to attempt to answer some of the questions not considered in Dence's study. This was necessary to confirm whether two distinct forms did exist and if not whether another explanation of the "dwarfs" was possible. Specifically, there were no age-size or morphological comparisons of the two forms and there was very little information concerning the biology of the normal-sized form.

## Materials and Methods

Wolf Lake, approximately 74°12′W and 44°01′N was selected for this study because of Dence's previous studies in the lake and because this lake, located on the Huntington Wildlife Forest Experiment Station property, has been maintained as a forest preserve and is relatively undeveloped. The bathymetric map (Heady 1942) indicates that it is an elongate, rectangular lake with a basin at the south end of 12 m maximum depth, a central basin of 14 m maximum depth, and a shallower north end with an approximate average depth of 6 m. The lake has a surface area of 59.8 ha and a maximum depth of 14 m.

Fish were sampled May 29–30 and October 9–12, 1971, and June 2–5, 1972. Fish were collected from spawning streams using seines and from the lake using gillnets and small-mesh trapnets (Beamish 1973a). Trapnets were set in many locations in an attempt to sample a variety of habitats. Gillnets of stretched-mesh size ranging from 1.9 to 10 cm were set in the deeper and shallower areas of the lake. Because immature fish and individuals representing the larger "subspecies" were difficult to find, all fish smaller than 100 mm and all those larger than 239 mm were weighed; fork length, sex, and maturity were recorded; and a scale sample and the first 4–6 anterior pectoral rays were collected for the determination of age as described by Beamish (1973b). Subsamples were selected by dividing total catch into smaller lots and sampling all fish in any one lot. Lots of fish of sizes 100–239 mm were subsampled from the catch to provide age-growth and age-maturity estimates of lake-dwelling and stream-spawning fish. Samples of all the large fish, subsamples of dwarf forms captured in the lake and all of the dwarf forms captured in the spawning streams at the south end of the lake were preserved in 10% Formalin for subsequent morphometric comparisons. A total of 93 specimens ranging from 88 to 518 mm total length were examined for meristics and proportions. Fish were selected to include representatives of each sex from the complete total length range as follows: 20 unsexed $< 100$ mm; 10 male and 10 female 101–200 mm; 10 male and 10 female 201–300 mm; 15 female $> 301$ mm; and 10 male and 8 female from fish found spawning in one of the creeks.

The following morphometric characteristics, except where noted, were determined according to the methods outlined in Hubbs and Lagler (1964); principal dorsal rays, principal anal rays, pectoral rays, pelvic rays, lateral line scale count (all scales from operculum to end of scales), scales above lateral line, scales below lateral line, branchiostegal rays (left side only), gill rakers on outer surface of first arch (number on upper and lower limb separated), gill rakers on inner surface of first arch (total only), fork length, total length, least depth of caudal peduncle, length of caudal peduncle, height of anal fin (origin to tip of longest ray), length of pectoral fin, length of pelvic fin, head length, snout length, and length of orbit.

The following proportions were calculated arithmetically: height of anal fin into total length, length of pectoral fin into total length, length of pelvic fin into total length, head length into total length, snout length into head length, length of orbit (eye diameter) into head length, caudal peduncle length into total length, and caudal peduncle depth into caudal peduncle length.

Factors for converting total length and fork length were calculated using the 93 specimens between 88 and 518 mm total length (Table 2). All lengths measured in the field were fork lengths and all lengths for meristic and morphometric series were total lengths.

## Results

Throughout the study 4250 white suckers were captured from the lake and 70 from a group of spawning individuals in the south inlet stream. All of the 70 inlet spawners and 1130 individuals from the lake were sampled completely for length, weight, sex, maturity, and age. An additional 400 fish captured in the lake were sampled for length, weight, sex, and maturity. Fish larger than 199 mm were sorted and sampled from

TABLE 1. Relative size and sex ratios of white suckers (*Catostomus commersoni*) from Wolf Lake. Numbers in parentheses are numbers of individuals in the samples.

| Length-class (*mm*) | % of catch in each length-class: Gillnet[a] plus trapnet (4044) | Subsample from trapnets[b] (2820) | South inlet spawning individuals | Sex ratio[c] ♀ : ♂ | No. of ♂[c] |
|---|---|---|---|---|---|
| 0–199 | 83.2 | 87.2 | 97.1 | 0.94:1 | 449 |
| 200–219 | 10.8 | 8.3 | 2.9 | 3.25:1 | 40 |
| 220–239 | 3.3 | 3.2 | 0 | 6.13:1 | 15 |
| 240–279 | 1.8 | 1.0 | 0 | 22.00:1 | 3 |
| 280–299 | 0.3 | 1.0 | 0 | No ♂ | 0 |
| 300–399 | 0.3 | <0.1 | 0 | No ♂ | 0 |
| ≥400 | 0.3 | 0.1 | 0 | No ♂ | 0 |

[a]Total gillnet catch 775 individuals. Effort expressed as meters of gillnet set for a 24-h period.

| Stretched mesh (*cm*) | 1.9 | 3.2 | 3.8 | 4.5 | 5.0 | 6.5 | 7.5 | 10.0 | 11.5 |
|---|---|---|---|---|---|---|---|---|---|
| Meters gillnet | 60 | 60 | 200 | 170 | 75 | 170 | 365 | 90 | 275 |

[b]A total of 20 trapnets were fished for a 24-h period during the study and the total catch was 3545 individuals.

[c]Sex could not be determined for all 1600 fish examined.

4050 of all fish captured. Only 680 or 16.8% were larger than 199 mm and only 6% were larger than 219 mm (Table 1). The actual percentage of fish larger than 199 and 219 mm in the population may be slightly lower and closer to the values obtained from the trapnet samples (Table 1) because gillnetting operations tended to capture larger individuals, e.g. one 5-cm stretched mesh gillnet captured white suckers ranging in length from 200 to 290 mm. Less than 3% of the inlet spawners were larger than 199 mm and none was larger than 219 mm (Table 1).

The sample of fish smaller than 200 mm contained approximately equal numbers of male and female individuals (Table 1). Approximately 94% of fish larger than 199 mm were females and all 35 fish in length-classes above 279 mm were females. Only 101 females and 3 males larger than 239 mm were found from all fish sampled. The largest male measured 262 mm.

Fish first started to mature at age 3 (Fig. 1). At age 4, 48% of the fish examined were mature and by age 5 only 13% were still immature. Males dominated the catch of mature fish to age 6 and after age 6, females were more numerous (Fig. 1). Sex could not always be distinguished in immature fish, especially in age 1 and 2 individuals. Of the fish that could be sexed, 54% of 77 age 3 immature fish were females; 61% of 38 age 4 immatures were females; 83% of 18 age 5 immatures were females; and 93% of 14 immature white suckers older than age 5 were females. Growth after maturity often is reduced and fish that mature at older ages might also be larger. However, an examination of immature fish 4 and 5 yr old showed that immature fish were actually smaller ($t$-test $P \leq 0.05$) than mature fish. There also was no indication from the length-frequency distributions of immature fish that two size-groups existed with age-classes.

The growth curves for fish taken in the sampling periods were similar. Only the curve for the spring 1972 sampling period is presented (Fig. 2). Since very few larger and older fish were captured, the individual ages of all fish captured during the study that were age 10 or older were included in Fig. 2. Some of the larger females reached ages up to 18 yr, whereas the oldest and largest male was 14 yr. The probability that the female growth curve was significantly different from the male growth curve (Fig. 2) was tested by estimating the probability that mean female size was larger than mean male size for each age-class of mature fish ($t$-test). The probabilities were combined for each sex by means of a chi-square transformation (Fisher 1950, Section 21.1) and it was found that the growth curves in Fig. 2 were significantly different ($P < 0.05$), indicating that female growth was greater than male growth after age 3.

A catch curve is a graph of log frequency against age (Ricker 1958). From the slope of the descending right limb of the curve it is pos-

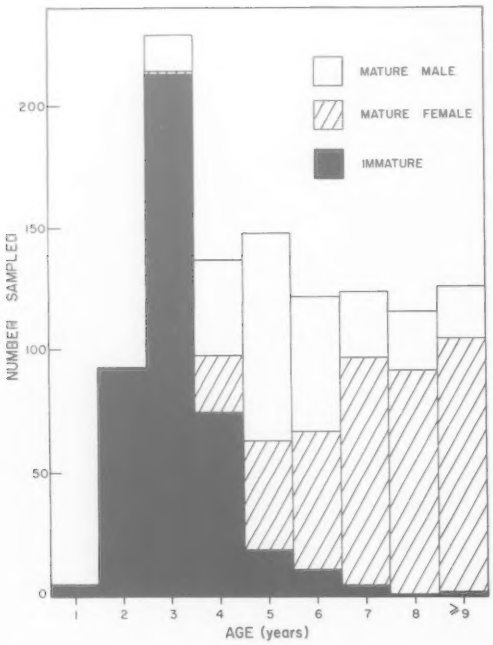


FIG. 1. Relative numbers of mature males, mature females, and immature white suckers (*Catostomus commersoni*) sampled for age in Wolf Lake.

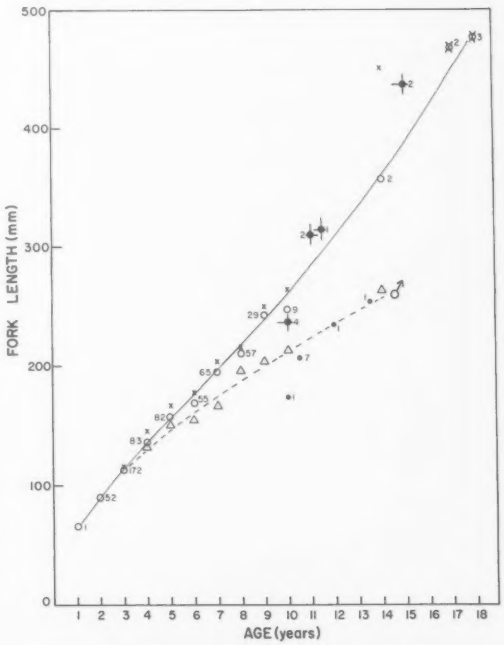


FIG. 2. Growth curve for white suckers in Wolf Lake; curves fitted by eye: ○, mean fork length; ×, mean form length females; sampled in the spring 1972; △, mean fork length males; sampled in the spring 1972; ◆, mean fork length females from all other sampling periods; ●, mean fork length males from all other sampling periods.

sible to estimate the natural mortality in a population. The catch curve of a total trapnet catch of 286 individuals (Fig. 3) showed that age 4 males suffer a higher mortality than females. Not until age 7 do females incur a rate of mortality similar to males. The ratio of females to males captured for this sample was 1.8:1 and was slightly lower than the ratio of 2.2:1 for the total number of fish that were sampled for sex throughout the study (Table 1). This indicates that this trapnet catch could be considered a representative sample of the population.

The meristic and morphometric measurements (Table 2) were similar to the description of those of the common or normal-sized white sucker (Scott and Crossman 1973). The number of anal and branchiostegal rays were seven and three, respectively, in all fish. In none of the characters examined could a definite separation be made between the small and large forms. A discriminant function analysis was applied to the meristic data of Wolf Lake white suckers to determine if a separation of two forms was possible. Fish larger than 220 mm fork length or 239 mm total length were considered to be normal-sized according to the separation used by Dence (1937). No significant difference in the meristic measurements of 23 normal-sized and 48 smaller-sized suckers was found ($P \leqslant 0.05$). In addition to the discriminant function analysis all characters in Table 2 were arranged by total length and the means of the smallest 47 were compared with the means obtained from the largest 46 using a *t*-test. Tests were made for males and females separately and combined. Significant differences ($P \leqslant 0.05$) were found for arc sine transformed proportion values involving snout length to head length, eye diameter to head length, length of pectoral and pelvic rays, pectoral and pelvic fin-ray counts, inner gill raker counts for males and females, outer gill raker counts for females, and the number of scale rows to the origin of the anal fin in females. However, snout length, head length, eye diameter, pectoral and pelvic fin lengths were very strongly correlated to total length (linear regression, $r = 0.94$–$0.99$) indicating that these measurements were part of a continuous series and not related to any size-group. Thus, only the inner gill raker counts for

TABLE 2. Meristic and morphometric summary. Total length = 1.09 × fork length; ≤195-mm length-class excludes all unsexed fish under 100 mm.

| | < 100 m | 101–200 m | | 201–300 m | | 300 + m | Creek spawners | | ≤195 mm | | > 195 mm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂ + ♀ | ♂ | ♀ | ♂ | ♀ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | Total |
| Pectoral rays | 17.1 | 16.6 | 16.9 | 16.9 | 17.0 | 14.9 | 18.1 | 16.9 | 17.1 | 17.0 | 17.3 | 15.9 | 16.7 |
| Pelvic rays | 9.4 | 9.9 | 9.9 | 10.0 | 9.6 | 9.1 | 10.1 | 9.8 | 9.9 | 9.8 | 10.0 | 9.8 | 9.6 |
| Dorsal rays | 11.2 | 11.3 | 11.4 | 11.0 | 11.5 | 11.0 | 11.3 | 11.6 | 11.4 | 11.3 | 11.3 | 11.3 | 11.2 |
| Scale rows lateral line to dorsal | 10.0 | 11.1 | 11.3 | 11.0 | 10.4 | 11.5 | 10.3 | 10.4 | 10.8 | 11.1 | 10.8 | 10.9 | 10.7 |
| Scale rows lateral line to origin of anal fin | 7.3 | 8.1 | 7.4 | 8.2 | 8.6 | 8.3 | 8.1 | 7.8 | 8.0 | 7.6 | 8.2 | 8.1 | 7.9 |
| Gill rakers inner | 24.7 | 25.7 | 24.5 | 26.5 | 25.4 | 25.8 | 26.0 | 22.3 | 23.6 | 25.1 | 26.6 | 25.8 | 25.5 |
| Gill rakers outer | 21.8 | 21.6 | 21.3 | 23.4 | 22.4 | 22.7 | 22.4 | 26.6 | 22.1 | 21.5 | 22.8 | 22.6 | 22.2 |
| Lateral line scales | 62.6 | 60.3 | 63.8 | 63.6 | 65.3 | 65.7 | 64.2 | 66.4 | 62.0 | 64.3 | 63.5 | 65.7 | 63.9 |
| Height anal fin: total length | 0.147 | 0.205 | 0.165 | 0.210 | 0.169 | 0.158 | 0.208 | 0.165 | 0.203 | 0.165 | 0.210 | 0.163 | 0.174 |
| Length pectoral fin: total length | 0.160 | 0.173 | 0.167 | 0.175 | 0.166 | 0.160 | 0.173 | 0.165 | 0.172 | 0.167 | 0.174 | 0.162 | 0.166 |
| Length pelvic fin: total length | 0.116 | 0.141 | 0.120 | 0.152 | 0.113 | 0.110 | 0.142 | 0.111 | 0.141 | 0.109 | 0.157 | 0.112 | 0.125 |
| Head length: total length | 0.213 | 0.203 | 0.217 | 0.203 | 0.207 | 0.214 | 0.202 | 0.212 | 0.203 | 0.210 | 0.203 | 0.211 | 0.210 |
| Snout length: head length | 0.430 | 0.454 | 0.448 | 0.484 | 0.502 | 0.500 | 0.488 | 0.513 | 0.463 | 0.464 | 0.481 | 0.519 | 0.471 |
| Eye diameter: head length | 0.210 | 0.188 | 0.180 | 0.170 | 0.164 | 0.136 | 0.175 | 0.166 | 0.182 | 0.177 | 0.172 | 0.150 | 0.174 |
| Caudal peduncle length: total length | 0.144 | 0.155 | 0.160 | 0.152 | 0.148 | 0.154 | 0.149 | 0.154 | 0.153 | 0.156 | 0.151 | 0.153 | 0.151 |
| Caudal peduncle depth: caudal peduncle length | 0.500 | 0.477 | 0.474 | 0.504 | 0.493 | 0.484 | 0.475 | 0.478 | 0.475 | 0.490 | 0.494 | 0.486 | 0.488 |

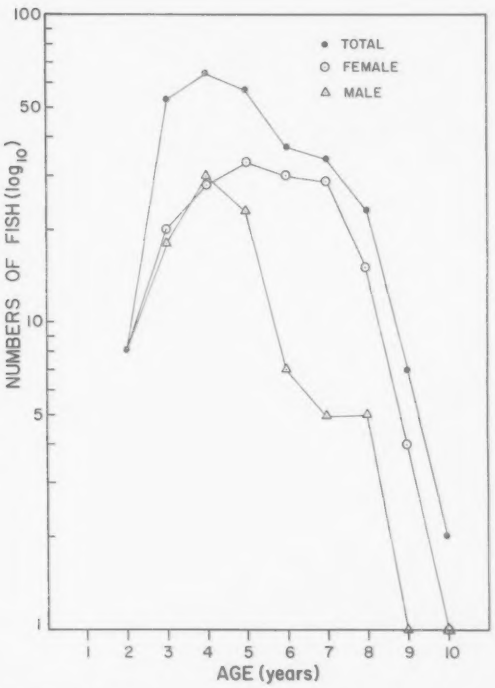


FIG. 3. Catch curve for one total trapnet catch.

males and females, the outer gill raker counts for females, the number of scale rows to the origin of the anal fin, and the female pectoral ray counts could be considered to be significantly different between the two groups. In all but one case the frequency counts (Table 3) were similar and overlapped to the extent that no distinction between size-groups could be made. Only when comparing the number of pectoral rays for females larger than 330 mm total length with fish smaller than 196 mm total length was there any apparent separation between the groups. However, there still was some overlap in counts, especially for the intermediate lengths, and this character was only significant for females. Thus, a separation of the two forms could not be made using the meristic or morphometric characteristics.

## Discussion

Small and larger mature white suckers still exist in the lake in which the biology of the so-called dwarf white sucker was originally studied. However, there was one continuous size range and no evidence was found to indicate two separate populations of different-sized individuals. Immature fish of both sexes were equally abundant and similar in size up to age 3. At age 3 a greater percent of males began to mature and after age 4, males incurred a higher mortality rate and slower growth than females. As a result there was a disproportionate number of larger and older females in the population. This culminated in the extreme condition that 100% of the fish larger than 279 mm fork length were females. Also important was the observation that these large individuals constituted <1% of the catch. Thus, the large- or normal-sized white sucker was not numerous; indeed, such large fish must be considered to be rare.

Dence (1937) stated that the size range for mature normal white suckers in Wolf Lake was 12-18 in., standard length, and for mature dwarf

TABLE 3. Frequencies of counts of characters that differed significantly ($P \leq 0.05$) between the large and small size-classes.

| Character | Size range total length (*mm*) | Mean of frequency count | Frequency | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6 | 7 | 8 | 9 | 10 | | | | | | |
| No. scale rows | 88–195 | 7.7 | 0 | 4 | 7 | 0 | 0 | | | | | | |
| lateral line to anal fin (♀) | 196–518 | 8.1 | 0 | 3 | 22 | 4 | 1 | | | | | | |
| | <100, sexes combined | 7.3 | 1 | 14 | 4 | 1 | 0 | | | | | | |
| | >330[a] | 8.3 | 0 | 0 | 10 | 2 | 1 | | | | | | |
| | | | 15 | 16 | 17 | 18 | 19 | | | | | | |
| No. pectoral rays (♀) | 88–195 | 17.0 | 0 | 2 | 7 | 2 | 0 | | | | | | |
| | 196–518 | 15.9 | 5 | 9 | 12 | 2 | 0 | | | | | | |
| | <100, sexes combined | 17.1 | 0 | 1 | 14 | 4 | 0 | | | | | | |
| | >330[a] | 15.6 | 5 | 5 | 1 | 0 | 0 | | | | | | |
| | | | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
| No. outer gill rakers (♀) | 88–195 | 21.5 | 1 | 0 | 5 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 196–518 | 22.5 | 0 | 1 | 3 | 10 | 7 | 4 | 0 | 1 | 0 | 0 | 0 |
| | <100, sexes combined | 21.8 | 1 | 2 | 3 | 8 | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| | >330[a] | 22.3 | 0 | 0 | 2 | 2 | 3 | 1 | 0 | 1 | 0 | 0 | 0 |
| No. inner gill rakers (♀) | 88–195 | 24.8 | 0 | 0 | 0 | 1 | 1 | 3 | 1 | 4 | 1 | 0 | 0 |
| | 196–518 | 25.9 | 0 | 0 | 0 | 2 | 1 | 4 | 2 | 4 | 9 | 3 | 1 |
| | <100, sexes combined | 24.7 | 1 | 0 | 0 | 1 | 0 | 5 | 6 | 4 | 2 | 0 | 0 |
| | >330[a] | 25.7 | — | — | — | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| (♂) | 88–195 | 25.4 | 0 | 0 | 1 | 0 | 2 | 1 | 5 | 4 | 1 | 2 | 0 |
| | 196–518 | 26.6 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 4 | 2 | 2 |
| | <100, sexes combined | 24 7 | 1 | 0 | 0 | 1 | 0 | 5 | 6 | 4 | 2 | 0 | 0 |
| | >330[a,b] | — | — | — | — | — | — | — | — | — | — | — | — |

[a]Minimum total length for normal white sucker as defined by Dence (1937).
[b]No males were >330 mm total length.

suckers, 5–8 in. Allowing an increase in length of approximately 10% to convert to fork length this defines mature common suckers as ranging from 330 to 500 mm in fork length and dwarf white suckers from 140 to 220 mm. According to these limits all common white suckers captured during the present study were females. If all fish larger than 220 mm fork length were considered to be the normal-sized subspecies, then the population of common white suckers examined in the present study would still be composed mostly of females. Only 3 of the 101 fish larger than 239 mm fork length from a sample of 4320 individuals were males. The larger females examined in early June 1972 had spawned and since larger males were extremely rare, these fish probably spawned with the males from within the size range Dence considered to be the dwarf subspecies.

Smaller fish of each sex were also younger fish. There was no indication that the lake contained discrete groups of similar-aged individuals that differed greatly in size. The examination of immature fish did not reveal two size-groups of immature fish. In many populations of normal-sized white suckers growth tends to be curtailed after the fish mature (Beamish 1973b). The Wolf Lake population did not demonstrate this pronounced reduction in growth after maturity. The larger fish result from the rare occurrence of a female surviving and continuing growth up to the maximum age observed for the species. The small fish that characterize the inlet and outlet streams during spawning apparently represent the segment of the population that is able to navigate in the restrictive openings and channels of these streams.

This interpretation differs from Dence's (1948) explanation because this study concentrated on the period of lake residence and because the fish

could be accurately aged using sections of pectoral fins. The ages assigned were consistent with the size of young-of-the-year, age at first maturity, and growth as estimated by Dence (1948). Dence (1948) did not conduct extensive studies in the lake as he was reluctant to catch trout. His 1948 report indicated that he captured only 12 large or common white suckers from a sample of 12,000 fish. Had he conducted such a study we feel he would have realized that the larger form was rare and composed mostly of females.

An alternative explanation that the structure of the population has changed since Dence's study and the present study does not appear probable. The lake and watershed have been preserved in their natural state as part of a forestry research area. The species composition of the lake found in this study (Beamish 1973a) was similar to that recorded by Dence (1937, 1940). His observations (1948) that females started to mature at a fork length of 140 mm, 12–25 mm larger than males, was similar to observations in this study. Similarly, his observation that several male white suckers tagged during their 1st yr of maturity and recaptured after 7 yr had shown increases in length of 75 mm is similar to a mean increment of 90 mm found in this study for a similar period (Fig. 2). Finally in his one netting attempt for the 1948 study he reported (p. 92) that a 1¼-in. bar mesh trammel net resulted in a catch of mostly females. He acknowledged that all fish less than about 200 mm fork length could pass through the net. Thus, the disproportionate number of larger female fish was not unique to this study.

From the large number of fish captured by Dence (1948) during his study and the facility of capture of individuals during this study (Beamish 1973a) Wolf Lake apparently contains a large number of white suckers for such a small lake. It is suggested that a large percentage of the population is composed of small individuals that do not attain older ages or larger sizes as a result of environmental pressures. The subspecies-separating character used by Dence (1948) that the common form spawned before the dwarf form is consistent with our personal observations that larger individuals within a white sucker population are the first to spawn.

Definitions of subspecies do not require a complete morphological separation of forms and the failure of the tests used in this study to isolate the two forms meristically or morphometrically do not by themselves indicate that no separation exists at the subspecies level. There may be other characters that should have been examined; there may also have been measuring problems associated with the temperature during or the timing of the development of characters chosen. However, when the negative results of the meristic and morphological data analysis are considered in association with the age, size, and sex composition of the population, there can be little doubt that distinct dwarf and normal-sized subspecies do not exist in this lake. Considering the results of this study, the observation by Dence (1948) that considerable variation in size of dwarf white suckers may occur within the same watershed, and that variation in size at maturity occurs throughout the range of the species (Beamish 1973b), we conclude that the occurrence of a distinct dwarf subspecies has not yet been demonstrated.

## Acknowledgments

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# Modèles linéaires de prédiction des débarquements de homard aux Iles-de-la-Madeleine (Golfe du Saint-Laurent)

F.-ROBERT BOUDREAULT, J.-NORMAND DUPONT[1] ET CLAUDINE SYLVAIN[2]

*Direction générale des Pêches maritimes, Ministère de l'Industrie et du Commerce, Québec, Québec G1P 3W8*

BOUDREAULT F.-R., J.-N. DUPONT ET C. SYLVAIN. 1977. Modèles linéaires de prédiction des débarquements de homard aux Iles-de-la-Madeleine (Golfe du Saint-Laurent). J. Fish. Res. Board Can. 34: 379–383.

Les débarquements annuels (1912–74) de homard des Iles-de-la-Madeleine, peuvent être représentés, en utilisant la méthode de Box et Jenkins, par un modèle autorégressif d'ordre un qui explique 41,9% de la variance totale. Si nous analysons l'effort de pêche et la température superficielle, nous obtenons, par une régression sur les composantes principales, un modèle linéaire contenant le premier modèle et faisant intervenir la température de décembre, il y a 8½ ans et celle de l'hiver d'il y a 3½ ans. Ce modèle de prédiction explique 84,4% de la variance totale. L'interprétation biologique est faite comme suit: les débarquements annuels dépendent du stock disponible, de l'influence de la température sur la probabilité du survie des larves et, possiblement, de cette même influence sur la probabilité de passage à la maturité sexuelle de la classe d'âge de 5 ans.

BOUDREAULT F.-R., J.-N. DUPONT ET C. SYLVAIN. 1977. Modèles linéaires de prédiction des débarquements de homard aux Iles-de-la-Madeleine (Golfe du Saint-Laurent). J. Fish. Res. Board Can. 34: 379–383.

Annual lobster landings (1912–74) at the Magdalen Islands can be represented with the Box and Jenkins method, by a first order autoregressive model which explains 41.9% of the total variance. Analysis of fishing effort and surface temperature yields, through a regression on principal components, a linear model that includes the first and makes use of the December temperature 8½ yr ago and that of the winter 3½ yr ago. This prediction model explains 84.4% of the total variance. The biological interpretation is as follows: annual landings depend on available stock, on the influence of temperature on the survival probability of larvae and possibly, on a similar influence on the probability of access to sexual maturity of the 5-yr-old class.

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## Objectif

NOTRE objectif est la prévision du potentiel exploitable du homard, une ressource renouvelable et commercialement importante au Québec.

Pour obtenir ce modèle de prédiction nous avons voulu, dans un premier temps, voir jusqu'à quel point les observations les plus fréquemment disponibles, soit les débarquements annuels, étaient suffisantes ou utiles aux fins de gestion d'une pêcherie.

Dans un second temps, nous avons voulu faire les meilleures prédictions possible, en examinant le rôle de l'effort de pêche et de la température.

[1]Direction générale des Eaux, Ministère des Richesses naturelles, Québec, G1S 4N6.

[2]Direction générale des Systèmes de Gestion, Ministère de la Fonction publique, Québec, G1R 4Z9.

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## Modèles à une seule variable

La série que nous désirons modeler est celle des débarquements de homard aux Iles-de-la-Madeleine pour les annés 1912 à 1974 (fig. 1). Ces valeurs proviennent de Bergeron (1967) pour la période 1912–64 et du Bureau de la Statistique du Québec pour les années subséquentes.

La méthode de Box et Jenkins (1970) permet d'établir pour les séries chronologiques, des modèles généraux, linéaires par rapport aux coefficients (Draper et Smith 1966, p. 263 et seq.). Ainsi deux modèles sont retenus:

$$Z_t = 0{,}6\ Z_{t-1} + a_t \qquad (1)$$

et

$$Z_t - 0{,}58\ Z_{t-1} = a_t + 0{,}04\ a_{t-1} \qquad (2)$$

Ces deux modèles ne peuvent être départagés au niveau de l'analyse des résidus: autocorrélation des résidus, test portemanteau, périodogramme cumulatif, statistique de Bartlett (Sylvain et Boudreault 1976).

Dans un souci de parcimonie, nous ne retenons que le modèle autorégressif d'ordre un (éq. 1), le

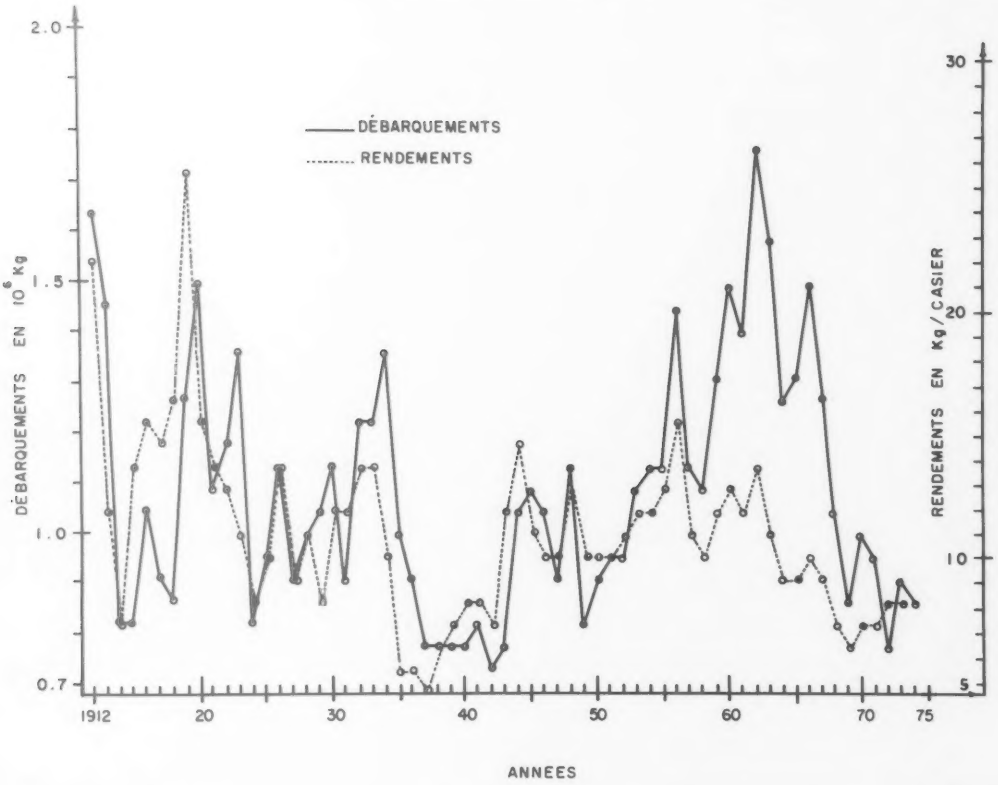


FIG. 1. Série temporelle des débarquements et des rendements annuels de homard aux Iles-de-la-Madeleine (1912–74).

paramètre moyenne mobile du second modèle étant très petit (Sylvain et Boudreault 1976, p. 20).

Ce modèle, bien que justifié, n'est pas satisfaisant, puisqu'il n'explique que 41,9% de la variance de la série. Pour un si petit nombre d'observations, cette méthode fournirait de meilleurs résultats, si notre série était plus homogène (Sylvain et Boudreault 1976, sec. 2.5) ou avait un plus haut degré de stationnarité (Reid 1969; Kendall 1971; Chatfield et Prothero 1973; Roy 1976).

Nous avons fait un second essai portant sur la série des rendements (capture ÷ effort de pêche). Cette série est plus riche en information, puisqu'elle tient compte d'une autre variable, l'effort de pêche, mesurée par le nombre de casiers utilisés par les pêcheurs au début de la saison. Notons que cette série comporte le même nombre d'observations et a même allure que celle des débarquements (fig. 1).

Le modèle résultant de cet essai est équipollent au précédent, même en ce qui concerne le pourcentage de variance expliquée (Sylvain et Boudreault 1976). Nous sommes donc amenés à conclure que les succès de cette méthode dépendent davantage du nombre d'observations que de la stationnarité de la série.

Finalement, nous avons étudié une troisième série, les rendements-300. Ce souci vient du fait que le nombre de casiers utilisés par les pêcheurs au début de la saison ne tient pas compte a) du fait que les pêcheurs utilisent un nombre décroissant de casiers, à mesure que la saison de pêche progresse, b) que le nombre de jours de pêche peut être variable d'une année à l'autre, c) que la perte de casiers par suite de tempête peut limiter le nombre de casiers disponibles, et d) qu'une partie des casiers ainsi perdus capturent et retiennent du homard (Seldon et Dow 1975) diminuant ainsi les débarquements commerciaux.

Cette série des rendements-300 (fig. 2) est la meilleure évaluation dont nous disposions pour le rendement de la pêche du homard, car elle tient compte, spécifiquement, du nombre de jours de pêche et du nombre de pêcheurs utilisant, au début de la

saison, 300 casiers. Bien qu'elle ne comporte que 24 observations, cette série est nettement plus homogène que la précédente et la méthode de Box et Jenkins s'y révèle fort efficace puisqu'avec 3 fois moins d'observations, elle nous fournit le même modèle que dans les deux cas précédents. Bien qu'il soit aussi bien justifié, il demeure non satisfaisant, car il n'explique que 38,4% de la variance totale.

Nous concluons que cette méthode n'a pu fournir de modèle satisfaisant, vu la contrainte du nombre d'observations et corroborons ainsi l'opinion de Reid (1969, sec. 8) et de Box et Jenkins (1970).

## Modèle à plusieurs variables

Devant ce succès mitigé et pour en arriver à un meilleur modèle de prédiction, nous avons considéré en plus des débarquements ($d$), l'effort de pêche ($ep$) et la température ($t$) superficielle de l'eau de mer.

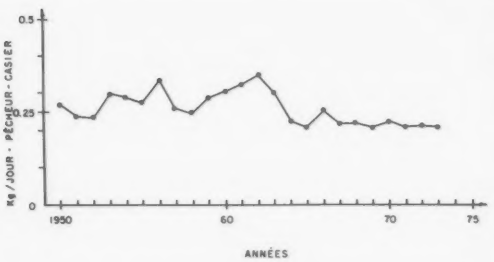


FIG. 2. Série des rendements-300 (1950–73).

Un régression sur les composantes principales devra être employée puisque les débarquements à prédire ($d_0$) dépendent des valeurs antérieures ($d_{-i}$) de ces mêmes débarquements (éq. 1).

Deux critères de décision ont présidé au choix des variables intéressantes et possibles. Ce sont, d'une part, l'examen de l'autocorrélation des débarquements et, d'autre part, les corrélations entre les variables disponibles et le débarquement annuel (Dupont et Boudreault 1976, sec. 1.4).

## Résultats

Bien qu'aucune règle stricte ne régisse le nombre nécessaire d'observations pour caler un tel modèle, nous le fixons arbitrairement à 5 par variable indépendante. Cet arbitraire, joint à la disponibilité des variables possibles, limite le modèle à trois variables indépendantes.

Afin de déterminer le meilleur modèle, nous allons considérer, par ordre, le nombre d'années d'observations et le coefficient de détermination.

Ayant trouvé les deux meilleurs modèles à une variable, les deux variables correspondantes sont retenues pour déterminer les deux meilleurs modèles à deux variables, et ainsi pour le modèle à trois variables (Draper et Smith 1966, sec. 6.1).

Le meilleur modèle ainsi dérivé des variables possibles, s'écrit:

$$\ln d_0 = 7{,}5055021 + 0{,}4792106 \ln d_{-1} + 0{,}0887181\ td_{-9} + 0{,}0417636\ td_{-4} \quad (3)$$

où le logarithme népérien des débarquements apparaît pour des raisons purement techniques.

Suivant les indications de Flowers et Saïla (1972), selon lesquelles la température moyenne de tout l'hiver d'il y a 9 ans devrait être utilisée. plutôt que celle de décembre ($td_{-9}$) seulement, nous avons successivement essayé a) ½ ($tm_{-8} + td_{-9}$), b) la température moyenne du 16 novembre au 15 décembre et c) celle du 1er au 15 décembre. Ces essais n'ont rien changé à la variance expliquée.

Par ailleurs, si nous remplaçons $td_{-4}$ par

$$th_{-43} = \tfrac{1}{2}\,(td_{-4} + tm_{-3})$$

le pourcentage de variance expliquée passe de 83 à 88,5%, pour les 14 années utilisées pour caler le modèle de l'équation 3.

En recalant ce modèle ainsi modifié avec le nombre maximal d'années disponibles soit, 18, le modèle

$$\ln d_0 = 8{,}8660071 + 0{,}3715854 \ln d_{-1} + 0{,}0812027\ td_{-9} + 0{,}0735824\ th_{-43} \quad (4)$$

explique 84,4% de la variance totale.

Pour ce modèle de prédiction, la matrice de corrélation présente une corrélation significative entre $td_{-9}$ et $\ln d_{-1}$; celle-ci apparaissait déjà dans le modèle de l'équation 3 (Dupont et Boudreault 1976, tableaux IV–V). Ceci apporte une seconde justification à l'emploi de la régression sur les composantes principales.

Ce modèle de prédiction double la variance expliquée par le modèle autorégressif (éq. 1).

Ce modèle permet de prédire les débarquements une année à l'avance et fournit une indication de la tendance pour les deux années subséquentes.

Pour 1976, les débarquements prédits sont de 1 832 000 lb de homard. Dans le mesure où cette prédiction se réalisera, les débarquements de 1977 atteindraient 2 048 000 lb et, dans les

mêmes conditions, ceux de 1978 seraient de 2 545 000 lb.

La régression multiple sur les composantes principales ne fournit pas d'intervalle de confiance.

## Interprétation

La première variable désigne les débarquements de l'année précédente ($d_{-1}$) et recoupe la suggestion de Scarratt (1964) selon laquelle il existe une relation entre le stock disponible et les débarquements annuels.

D'après nous, ceci provient du fait que le taux d'exploitation est approximativement constant, de l'ordre de 50 à 60%, et qu'en plus il n'y a vraisemblablement pas de surexploitation (J. Bergeron, communication personnelle, Service de Biologie, 2700 Einstein, Québec, G1P 3W8). Ainsi, une très bonne année ne devrait pas être suivie d'une très mauvaise année.

La seconde variable correspond à la phase pélagique, et, seules, les températures de décembre d'il y a 9 ans sont déterminantes pour la survie des larves.

En effet, la survie, de l'ordre d'environ 1% (Scarratt 1964), sera problématique pour les larves dont l'éclosion tardive ne leur permettra pas d'arriver au terme de la phase pélagique avant l'arrivée des températures inférieures à environ 3°C, alors que la mue deviendrait impossible (Templeman 1936).

Nous pouvons également considérer que les températures sous zéro suivies de la présence de glace rendent la survie fort improbable.

Dans un cas comme dans l'autre, les températures inférieures à 3°C ou sous zéro se retrouvent simultanément au cours de décembre. Aussi notre modèle ne contient-il que ces températures, et non celles de tout l'hiver.

Le temps réel écoulé entre la capture commerciale ($d_0$) et la température associée à la fin de la phase pélagique ($td_{-9}$), est de 8 ans et 1 mois, soit pour un homard dont l'âge réel, compté à partir de l'éclosion, est de 8¼ à 8½ ans. Ceci coïncide avec les estimations de l'âge du homard, à taille commerciale, telles que les donnent Wilder (1953) pour l'Ile-du-Prince-Edouard, Squires (1970), pour la côte sud-ouest de Terre-Neuve, et Corrivault et Tremblay (1948), pour la baie des Chaleurs.

La troisième variable serait reliée à l'époque de la maturité sexuelle et représente tout l'hiver qui précède de 3½ ans la capture commerciale. Il s'agirait donc de homards de 5 ans environ.

Cet âge doit être interprété comme l'âge moyen auquel la population commerciale atteint la maturité sexuelle. Bien que le rapport mâles/femelles soit généralement unitaire, les mâles croissent plus vite que les femelles (Corrivault et Tremblay 1948; Wilder 1953) et cette différence s'accentue lors de la maturité (Corrivault et Tremblay 1948; Templeman 1939). De même, l'âge, à la maturité sexuelle, est clairement fonction du sexe chez le homard. Dans la population de Homard du Maine, Krouse (1973) a observé que la longueur de carapace des femelles matures y est d'environ 90 mm, tandis que celle des mâles est d'environ 55 mm. Ceci correspondrait à des âges de 5¼ et 4 ans respectivement (Hughes et al. 1972).

Dans le cas du détroit de Northumberland, l'âge à la maturité serait de 4¼ ans environ, selon Wilder (1953). Comme nous connaissons l'influence marquée de la température sur la fréquence de la mue (Templeman 1936) et le fait que la température des eaux des Iles-de-la-Madeleine est effectivement inférieure à celle du détroit de Northumberland (Lauzier et Hull 1969), nous devons nous attendre que l'âge, à la maturité sexuelle, soit supérieur à 4¼ ans chez le homard des Iles-de-la-Madeleine. Notre modèle suggère qu'il atteint statistiquement sa maturité sexuelle vers 5 ans.

## Discussion

Nous ne retrouvons pas l'effort de pêche dans notre modèle de prédiction.

Comme le comportement temporel (fig. 1) et statistique de la série des rendements est similaire à celui des débarquements, nous en avions déduit que l'effort de pêche n'apportait aucune information supplémentaire.

Cependant, l'analyse de la série des rendements-300 montre l'importance d'une mesure plus appropriée, mais la brièveté de cette série a rendu impossible son utilisation dans notre modèle à plusieurs variables.

Nous en concluons donc que l'absence de l'effort de pêche dans notre modèle de prédiction est due à une mesure insuffisante plutôt qu'à la constance de cet effort, contrairement à ce que disent Flowers et Saïla (1972).

La présence, dans notre modèle de prédiction, des températures de décembre et de mai suggère l'utilité de connaître celles des mois de janvier à mars pour améliorer la troisième variable et, quoique ce soit peu probable, la seconde.

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Nous tenons à signaler la compétence et l'aide de J.-J. Bresse (décédé en février 1976), analyste à l'Université du Québec à Chicoutimi.

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Nous remercions le Bureau de la Statistique du Québec et le Service de Protection (Dir. Pêches marit.) du ministère de l'Industrie et du Commerce, pour avoir mis à notre disposition des statistiques inédites.

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# Genetics of Larval Tolerance to Reduced Salinities in Two Populations of Oysters, *Crassostrea virginica*

G. F. NEWKIRK, D. L. WAUGH, AND L. E. HALEY

*Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1*

NEWKIRK, G. F., D. L. WAUGH, AND L. E. HALEY. 1977. Genetics of larval tolerance to reduced salinities in two populations of oysters, *Crassostrea virginica*. J. Fish. Res. Board Can. 34: 384–387.

Oysters, *Crassostrea virginica* (Gemlin), were taken from a low- and an intermediate-salinity habitat. Males from each population were crossed with females from both populations and the offspring were raised at three salinities. Observations on survival during the larval period indicate that there are genetic differences between the two populations with regard to their tolerance to high and low salinity.

NEWKIRK, G. F., D. L. WAUGH, AND L. E. HALEY. 1977. Genetics of larval tolerance to reduced salinities in two populations of oysters, *Crassostrea virginica*. J. Fish. Res. Board Can. 34: 384–387.

Des huîtres, *Crassostrea virginica* (Gemlin), ont été prélevées d'un habitat de faible salinité et d'un autre de salinité intermédiaire. Les mâles de chaque population ont été croisés avec des femelles de l'une et l'autre population, et les descendants ont été élevés à trois salinités. La survie durant la période larvaire indique qu'il existe des différences génétiques entre les deux populations quant à leur tolérance à une forte et à une faible salinité.

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THE American oyster, *Crassostrea virginica* (Gemlin), is found in waters that differ greatly in salinity. Some are in virtually oceanic water whereas others are in very brackish water. Although previous studies have determined the survival and growth of oyster larvae at different salinities (Davis 1958; Davis and Calabrese 1964), there is little information regarding genetic differences in survival at reduced salinities. Davis (1958), using oysters from Long Island Sound (high salinity) and Hodges Bar, Maryland (low salinity), showed that there was no difference in survival between the larvae from these two populations when the animals were conditioned at the same salinity. However, when oysters were taken directly from Hodges Bar and spawned, the optimum salinity for larval survival was considerably less than that for the larvae from Long Island Sound oysters. This suggests a physiological, not a genetic, effect.

In the present work we have performed an experiment similar to that of Davis but we have made all possible crosses between a low-salinity population and a higher salinity population. If there is any genetic variation between populations regarding tolerance to reduced salinities, it should be detected in the performance of the offspring.

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## Materials and Methods

Oysters were collected from the upper part of the Richibucto River, Kent County, New Brunswick, in late June. This collection site, 32 km from the coast, is the upper limit of oysters in the river. These are low salinity (L.S.) oysters. At the time of collection there was a considerable depth of fresh water on the surface and the bottom water salinity was 9.5‰. These oysters were held in the laboratory at full salinity 30‰ at 18°C and fed *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* until spawning.

Oysters were also collected from Ostrea Lake, Halifax County, Nova Scotia. This is a brackish pond with a salinity that varies from about 20 to 25‰ through the year. The oysters were hatchery stock whose parents came from Prince Edward Island and though we do not know the original source population, we consider them to be intermediate salinity (I.S.) oysters. Due to space and food limitations in our lab conditioning unit, these oysters were left to develop gonads under natural conditions at the intermediate salinity of Ostrea Lake. The oysters were collected about a week prior to simultaneous spawning of both populations.

The oysters were spawned individually in beakers so that fertilization could be controlled. The pooled sperm for each population was used to fertilize the pooled eggs from each population. There were four crosses, two within population crosses and two between population or hybrid crosses. Each of the four crosses was replicated 4 times in water of 10 and

30‰ S and 8 times at 20‰ S. (The extra replications at 20‰ S were used later for another purpose.) The initial crosses were set up in pyrex beakers containing 1 $\ell$ of water with eggs at a density of 10 per ml. The beakers were screened every other day with a Nitex screen small enough to retain all larvae and new water was added. Penicillin and streptomycin were used at 81,250 and 22,200 IU/$\ell$, respectively. Larvae were fed a mixture of *Isochrysis galbana* and *Monochrysis lutherii* and held at 22°C. Larvae begin feeding at day 2 and some are ready to set soon after day 16. Samples were taken at these times and at a 6-day intermediate stage. Contents of each beaker was concentrated and a sample taken and counted to determine the number of surviving larvae.

## Results

The contribution of each sex in each of the two populations was determined for day 2. To do this consider the four crosses: I.S. ♀♀ × I.S. ♂♂, I.S. ♀♀ × L.S. ♂♂, L.S. ♀♀ × I.S. ♂♂, and L.S. ♀♀ × L.S. ♂♂. The contribution of a given sex from a certain population is the average of the number alive per millilitre in both crosses involving those individuals. As an example, the contribution of the I.S. ♀♀ is the average of the number alive per millilitre in the first and second crosses above. The results are given in Fig. 1. Offspring from both male populations had a much lower survival at 10‰ S than at higher salinities, but in comparing the two male populations the offspring of the L.S. males did better at 10‰ S but poorer at 20 and 30‰ S. Since males only contribute a nucleus to the larvae, there may be genes for salinity tolerance that are expressed in the first 2 days.

The results for the females appear to contradict the results from the males; but, the females produce eggs with considerable cytoplasm, resulting in the transfer of nongenetic maternal effects to the offspring. In the present experiment the I.S. females were conditioned at intermediate salinity and the L.S. females at high salinity. Thus, differences between males and females are due to maternal effects.

Survival between days 2 and 6 and between days 6 and 16 is considered to be the number alive on a given day (6 or 16) as a percentage of the number alive on the previous day (2 or 6).

A *G*-test on the number surviving during the time between day 2 and 6 shows there is a significant difference between the males and females of each population at all salinities except for the L.S. oysters at 10‰ S (Fig. 2). The opposite was true for the period between day 6 and 16; i.e. there was no difference between males and females except for the L.S. oysters and 10‰ S. For both periods the results of the L.S. oysters at

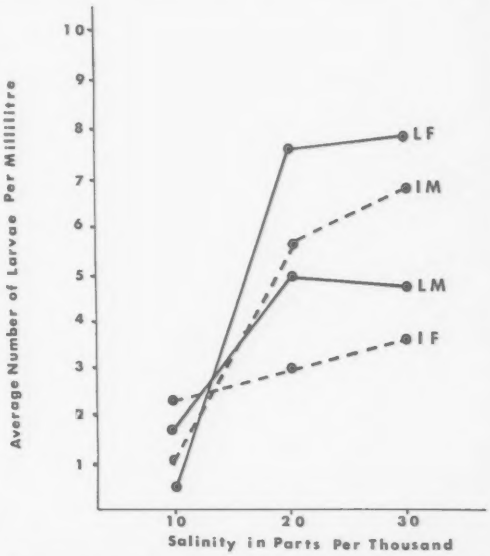


FIG. 1. Survival of oyster larvae from fertilization to day 2. Contribution of each sex in each population is graphed separately for three salinities. See the text for an explanation of how these components were calculated. LF = L.S. females; LM = L.S. males; IF = I.S. females; and IM = I.S. males.

10‰ S could be due to the small sample size of the females.

At day 2 the I.S. females produced larvae that have higher survival at low salinity than the L.S. females conditioned at high salinity. Comparing I.S. and L.S. females (Fig. 2) we see the same pattern for 2–6-day survival. The survival due to the males from 2 to 6 days contradicts the results at day 2. Apparently by the 6th day the I.S. males' offspring have a higher survival at low salinity than the L.S. males' offspring. Further analysis below will clarify this apparent contradiction.

The survival between day 6 and 16 shows that the nongenetic maternal effects were no longer present and the results for each sex within the same population were almost the same. Except for the L.S. oysters at 10‰ S, there was no statistical difference between the sexes, so the two sexes were pooled to give a value for the whole population (Fig. 3). When the two populations were compared at the same salinity the L.S. oysters did much better at 10‰ S, somewhat better at 20‰ S, and poorer at 30‰ S. *G*-tests on the survival show these differences to be highly significant. From this we conclude that there are genetic differences between these two populations for tolerance to different salinities.

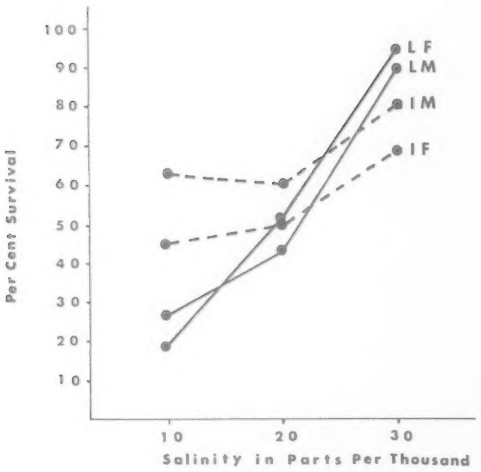


FIG. 2. Percent survival of oyster larvae from day 2 to 6 at three salinities. See Fig. 1 for explanation of symbols.

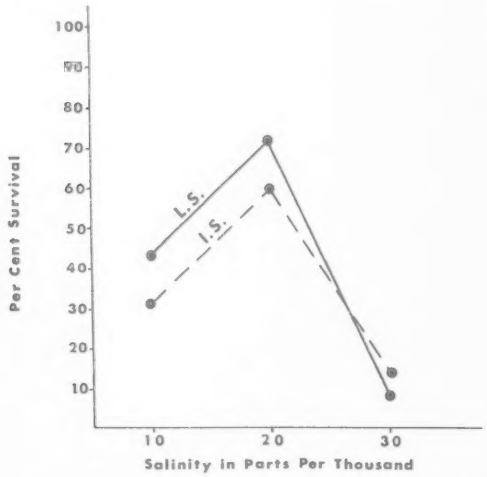


FIG. 3. Percent survival of larvae from day 6 to 16 at three salinities. Data are pooled for the two sexes in each population.

The results from each cross were examined separately to determine whether there is any 16-day survival difference between the hybrid crosses (L.S. ♀♀ × I.S. ♂♂, I.S. ♀♀ × L.S. ♂♂) and the within population (pure) crosses (I.S. ♀♀ × I.S. ♂♂, L.S. ♀♀ × L.S. ♂♂) (Fig. 4). At 10‰ S the hybrids do worse than the pure crosses. But there is a different pattern at the high salinities. An examination of Fig. 4 shows that the performance of the hybrid is not predictable from the performance of the pure crosses; e.g. at 10‰ S both hybrids have lower survivals than

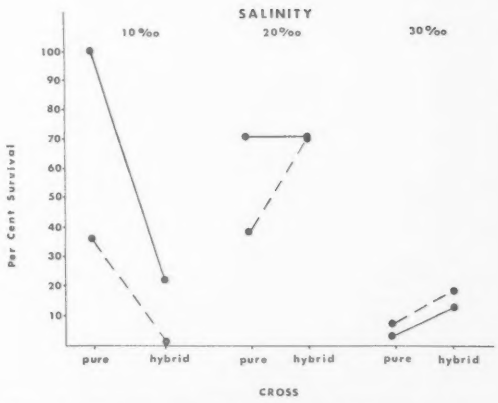


FIG. 4. Percent survival of oyster larvae from day 6 to 16 when males are crossed with females of the same population (pure) and with females of the other population (hybrid). Results for each cross are plotted separately for three salinities. Solid lines, L.S. males; broken lines, I.S. males.

either pure cross. In other words, there appear to be nonadditive effects, i.e. interactions. To test the significance of these interactions a three-way contigency table was set up for each salinity at 16 days. The three factors are: male population, female population, and mortality. The $G$ to test the significance of the male × female interaction was significant at $P = 0.05$ at salinities of 10 and 20‰, where there are obvious deviations of the hybrids from the pure crosses (see Fig. 4); the interaction was not significant at 30‰ although both hybrids had a higher survival than either pure cross. The lack of independence of the males and females is probably the result of genetic interaction.

## Discussion

Two factors appear to affect tolerance of oyster larvae to low-salinity water: a cytoplasmic and a genetic factor. The cytoplasmic factor is due to the conditions under which the eggs are formed. The composition of the egg will be affected by environmental factors, such as the salinity, as it develops. These factors, which are transferred from the mother to her offspring but are nongenetic, are referred to as maternal effects. This is demonstrated in the results from the L.S. oysters. They were conditioned (the gametes were developed) at high salinity and during early development the larvae showed high mortality at low salinity. However, later on, these same larvae showed much lower mortality at low salinity when presumably the larvae had had time to adjust osmotically and larval genes had taken over control of osmoregulation. The I.S. oysters

showed the opposite response. They had been conditioned at lower salinity, which resulted in lower mortality at low salinity. However, later on, mortality was high in low salinity compared with the L.S. oysters, presumably because the I.S. oyster larvae were not genetically as well adapted to low salinity as L.S. oysters.

The older the larvae, the more the performance of the individual is expected to be controlled by its genome and the less influence maternal effects have on determining the individual's phenotype. The effect of the larval genes can be seen when offspring from different males perform differently when reared under uniform conditions. We can assume that the cytoplasmic contribution of the male to a zygote is so small that the only effects seen will be those brought about by the genes he contributes. The larval oyster genes that affect tolerance to reduced salinity are expressed as early as the 2nd day (Fig. 1). During the period from day 2 to 6, however, there is no evidence of larval genes working but the maternal effects are still present. Between day 6 and 16 there is evidence of genes for salinity tolerance. The problem is to explain the appearance, disappearance, and reappearance of the expression of these genes. One explanation would be that there are two sets of genes which act at different times (when osmoregulation is critical). Another possibility is that the combination of the negative heterotic effect and the maternal effects is masking the expression of the genes from day 2 to 6. For example, based on the results at day 2 and the period 6–16 days, we would expect the larvae from L.S. males to have a higher survival than the larvae from I.S. males at 10‰ S during the period 2–6 days. However, the offspring of the I.S. males did better. This may be due to the offspring of the I.S. ♀♀ × L.S. ♂♂ doing poorly because of negative heterotic effects, while the offspring of the L.S. ♀♀ × L.S. ♂♂ did poorly because of maternal effects. The evidence for the negative heterotic effects being present shows up during the period 6–16 days. These effects are not clear during the period 2–6 days probably because of the importance of maternal effects. Thus, the apparent effect of the L.S. ♂♂ is that of low survival at 10‰ S. The result of this kind of interaction on the I.S. males would be to increase the percent survival of their offspring. The hybrid cross, L.S. ♀♀ × I.S. ♂♂, may do poorly because of negative heterotic effects, but the pure cross, I.S. ♀♀ × I.S. ♂♂, does reasonably well because of maternal effects. However, the result is a contradiction of the results at other times and the problem will only be resolved by controlling maternal effects.

Sometime in the period 6–16 days the maternal effects become unimportant and the results show that there is genetic differentiation of these populations for tolerance to reduced salinities. The fact that there was no difference between males and females at this time indicates that maternal effects are no longer present. In the meantime, there remains a difference between the effects attributable to each population (Fig. 3). This difference has to be attributable to genetic differences between the populations.

The presence of genotypic interactions was demonstrated in Fig. 4. If the action of the genes was simply additive, the hybrids would be intermediate to the two pure crosses. But this is not so. The hybrids respond differently, relative to the pure crosses, depending on the salinity, which indicates that there is also genotype–environment interaction (Falconer 1960). Since there was a common pool of genotypes placed at different salinities, the difference between the performance of the hybrids at different salinities must be moderated by the environment.

Although the I.S. oysters were actually taken from an area hundreds of miles from the L.S. oysters, they were, in fact, hatchery stock whose parents came from Prince Edward Island, not too far from the Richibucto River (source of the L.S. oysters); this should have little effect on the results. In the hatchery the mortality is much less than that experienced in the wild, so the individuals that survive probably contain a good sample of the genes of the wild parental stock. Thus, the genetic differentiation has apparently taken place over a distance easily covered by oyster larvae during the planktonic stage. How such differentiation is maintained is not clear. Probably, there is some habitat selection involved. Bivalve larvae are known to be discriminating in their choice of substrate and can in fact delay metamorphosis until they find a suitable substrate.

## Acknowledgments

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# Pleistocene Watershed Exchanges and the Fish Fauna of the Peel River Basin, Yukon Territory

R. A. BODALY AND C. C. LINDSEY

*Department of Zoology, University of Manitoba, Winnipeg, Manitoba R3T 2N2*

BODALY, R. A., AND C. C. LINDSEY. 1977. Pleistocene watershed exchanges and the fish fauna of the Peel River basin, Yukon Territory. J. Fish. Res. Board Can. 34: 388–395.

The Peel River basin is a unique Canadian glacial refugium containing many relict fish populations. Peel River is presently tributary to the Mackenzie River system, but at least twice during Pleistocene glaciations it was diverted into headwaters of the Yukon River system, offering the possibility of two-way transfer of aquatic organisms between the Mackenzie and Yukon. Present fish distributions in the Peel basin are summarized. Biochemical and morphological evidence suggests that races of at least six species now inhabiting the area (*Coregonus clupeaformis*, *Prosopium coulteri*, *Thymallus arcticus*, *Salvelinus namaycush*, *Esox lucius*, *Cottus cognatus*) originated from types which either came from the Yukon River system or developed in situ in unglaciated parts of the Peel.

BODALY, R. A., AND C. C. LINDSEY. 1977. Pleistocene watershed exchanges and the fish fauna of the Peel River basin, Yukon Territory. J. Fish. Res. Board Can. 34: 388–395.

Le bassin de la rivière Peel est un refugium glaciaire canadien unique contenant plusieurs populations relictuelles de poissons. La rivière Peel est présentement un tributaire du réseau du fleuve Mackenzie, mais à au moins deux reprises lors des glaciations du Pléistocène, elle a été détournée vers la tête des eaux du réseau du fleuve Yukon, permettant ainsi le transfert dans les deux sens d'organismes aquatiques entre le Mackenzie et le Yukon. Nous résumons la distribution actuelle des poissons dans le bassin de la rivière Peel. Des observations biochimiques et morphologiques donnent à croire que les races d'au moins six espèces habitant présentement la région (*Coregonus clupeaformis*, *Prosopium coulteri*, *Thymallus arcticus*, *Salvelinus namaycush*, *Esox lucius*, *Cottus cognatus*) eurent comme origine soit des types provenant du réseau du fleuve Yukon, soit des types qui se sont développés in situ dans les parties de la rivière Peel non soumises à la glaciation.

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THE unusual Pleistocene history of the Peel River system of central Yukon Territory in Canada has been of particular importance to the dispersal of freshwater organisms. The Peel basin was probably the only extensive unglaciated part of the present Mackenzie drainage, and formed part of the Bering refuge during the Pleistocene glacial advances. Peel River presently drains northeastward into the Mackenzie River, but it was blocked by ice at least twice during the Pleistocene and overflowed westward into the Porcupine River and thence to the Yukon River. Thus, the Peel has offered a potential route for faunal exchanges between the Yukon and Mackenzie River systems.

Many of the fish species found in the Peel basin exist over North America in two or more distinct racial types as a result of their separation by the Wisconsin ice mass. The Peel River system could presently be occupied by races originating from the Bering refuge in the unglaciated Yukon basin of Yukon Territory and Alaska, or by races originating from the Mississippi refuge south of the ice sheet in upper Missouri and/or Mississippi drainages (McPhail and Lindsey 1970).

This paper summarizes from geological evidence the probable sequence of flow reversals of Peel River. The known distributions of fishes in the area are described, and correlations are shown between their biochemical and morphological characters and their probable mode of entry into the region. The evidence suggests that the Peel region is unique in Canada in that it probably contains relict populations not only of fish but of other animals and plants. These populations are of great intrinsic interest but are peculiarly susceptible to destruction by man.

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## History of Pleistocene Drainage Changes

The following reconstruction of events during the last million years is based largely on publications and geological maps by Hughes (1969, 1972), Hughes et al. (1969), Prest (1970), Prest et al. (1968), and Bostock (1948). Our maps (Fig. 2) and inferences go beyond those of the author cited, and may well be wrong in detail, although we hope that the broad sequence of drainage reversals described is correct. For a similar treatment of Pleistocene drainage exchanges between the upper Yukon River and the Pacific, see Lindsey (1975).

At the onset of the Pleistocene, that part of the upper Porcupine River resembling a shepherd's crook (Fig. 1, inset) discharged eastward through McDougall Pass in the Richardson Mountains to Mackenzie River, instead of westward to the Yukon River as it does now (Fig. 2A). Few lake basins are likely to have existed following the very long preceding period without glaciations, but Tertiary or early Pleistocene downwarping in the Old Crow area probably helped to form the basins which subsequently filled with water.

Traces of two major glaciations are discernible in northern Yukon Territory. Which of these correlates with the (four) Pleistocene glaciations thought to have occurred farther south is uncertain. The most recent glaciation in our area is probably equivalent to the Late Wisconsin or Classical Wisconsin (McConnell and Kluane glaciations in southern Yukon Territory), and extended from roughly 30,000 to 10,000 yr before present (B.P.). The earlier and more extensive glaciation in our area may be equivalent either to the Early Wisconsin (Reid), ending about 55,000 B.P., or, more likely, to the still older Illinoian glaciation (Shakwak), ending about 230,000 B.P., which was the most extensive over most of North America. Although traces of the other glaciations visible to the south have been obliterated in our area, there may well have been more ice advances and watershed reversals than are described below.

The early (and most extensive) glaciation was a fusion of Laurentide ice moving northwest across the continent and Cordilleran ice moving north from the Mackenzie, Selwyn, and Ogilvie mountains. Ice covered most of the present Mackenzie River basin except for the Peel River, reached the eastern flank of the Richardson Mountains, and blocked the outlet of Porcupine River through McDougall Pass. A large, cold, deep lake backed up in the basins of the Eagle, Porcupine and Old Crow rivers, and began to discharge

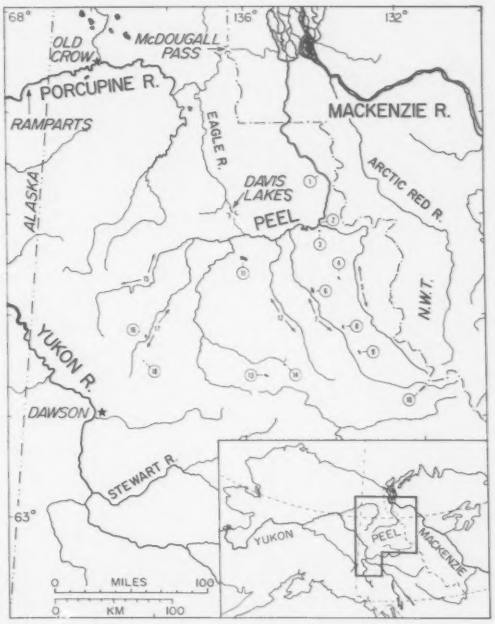


FIG. 1. Peel River and adjacent areas. Circled numbers are sites listed in Table 1.

westward into Yukon River at the Ramparts (Fig. 1, 2B). The eastern outlet of the Peel River was also blocked by an ice tongue; water from the unglaciated upper tributaries of the Peel became diverted northward into the head of Eagle River (and hence to the Yukon River) through a major channel presently occupied by Davis Lakes ("Palmer Lakes" of Hughes 1972).

When the early glaciation receded it reopened McDougall Pass and also the middle and lower Peel. The channels at the Ramparts and across Davis Lakes were abandoned, and the Porcupine and Peel rivers resumed their eastward flows into the Mackenzie. The main drainage pattern reverted to its preglacial condition (Fig. 2A), although there had doubtless been local changes brought about by the deposition of moraines and by cutting of ice-marginal channels.

At the onset of the last glaciation, the ice front again moved west to block McDougall Pass. The Porcupine River again backed up, forming a series of large lakes which discharged westward via the ramparts to Yukon River. Ice also blocked the lower Peel, but this time extended southwestward only to about the junction of the Peel and Snake rivers (Fig. 2C). An irregular lake backed up to fill middle Peel Valley ("Bonnet

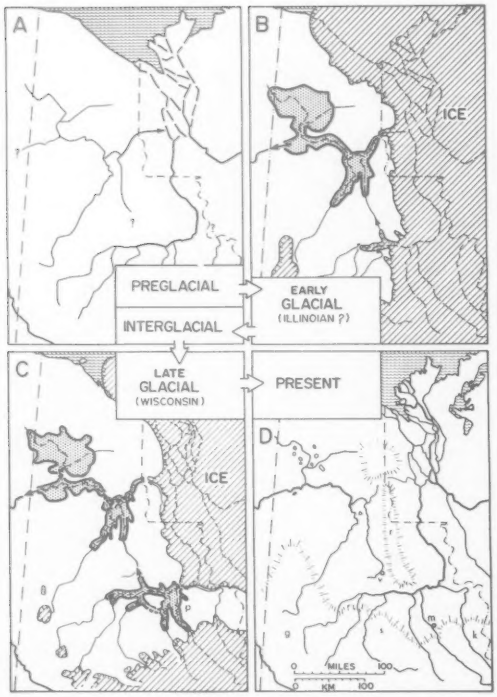


FIG. 2. Drainage patterns during successive Pleistocene stages; g — Ogilvie Mountains, k — Mackenzie Mountains, m — Margaret Lake, p — Bonnet Plume Basin, r — Richardson Mountains, s — Selwyn Mountains, v — Davis Lakes.

Plume Basin" of Hughes 1972) at least as far upstream as the junction of Blackstone and Ogilvie rivers. The lake rose until it overflowed north through the Davis Lakes channel and thence via Eagle River to the Yukon River.

During the last glaciation the westward outflow through the Ramparts eroded down until it reached a level below that of McDougall Pass. Therefore, when the ice retreated for the last time, the Porcupine River continued to drain westward into the Yukon (Fig. 2D). The Davis Lakes outlet from Bonnet Plume Basin was abandoned, and that lake drained away as its present eastward outlet via Peel River became ice free. All but remnants of the great glacial lakes in northern Yukon Territory have disappeared due to continued down-cutting of their drainages and deposition of sediments.

Today McDougall Pass forms a negligible divide between the tributaries to the Bell River (Yukon River system) and Rat River (Mackenzie River system). During high water periods the two are reputed to mingle. Thus, fish able to navigate small streams might breach the watershed divide under present conditions.

In the former discharge channel from Bonnet Plume Basin to Eagle River, Davis Lakes lie in the floor of the channel and drain northward (Fig. 1). Discharge from the Peel system through this channel must have ceased before the main Mackenzie system became ice free at the last ice recession. Therefore, fish species now present in Davis Lakes have either entered from the Yukon system via the northern lake outlet in recent times, or have persisted since the channel carried waters from Bonnet Plume Basin to the Yukon.

Margaret Lake (6 in Fig. 1; m in Fig. 2D) lies at an altitude of 490 m and was either included in or lay close to the lake which occupied Bonnet Plume Basin. Now the lake has no surface outlet and is perched more than 30 m above the trench of the Bonnet Plume River which passes at a distance of 1 km. Presumably fish species now present in Margaret Lake have persisted there since the recession of glacial lake waters at the close of the Wisconsin.

The only other lakes sampled that were probably close to or included in the waters of Bonnet Plume Basin were Dog and Hungry lakes (3 and 11 in Fig. 1). Postglacial access to both has been provided by surface outlets. All the other lakes in the Peel drainage shown in Fig. 1 lie well above the water level of Bonnet Plume Basin.

A recent study by Ford (1976) shows that an area of the Nahanni River basin (now tributary to the Liard–Mackenzie) escaped glaciation and contained proglacial lakes. These might conceivably have drained north and west to Bonnet Plume Basin during maximum glaciations, but little information is available yet on the Pleistocene history of the intervening area.

## Materials and Methods

Table 1 lists the known occurrence of fish species in all sites collected in the Peel River system in Yukon Territory. Most sites were visited by at least one of us between 1960 and 1975. The Davis (Palmer) Lakes at the headwaters of Eagle River (Porcupine system) were sampled by us in 1975. Lake whitefish specimens from the Mackenzie delta (Middle Channel near Inuvik) were obtained commercially. For records of additional species which have been taken in the lower reaches of Peel River in Northwest Territories, see McPhail and Lindsey (1970), Stein et al. (1973), and Jessop and Lilley (1975).

Lactate dehydrogenase (*ldh*: L-lactate: NAD oxidoreductase, EC 1.1.1.27) and glycerol-3-phosphate dehydrogenase (G-3-PDH: L-glycerol-3-phosphate:

TABLE 1. Fish species recorded at Peel River basin sites within Yukon Territory. Sites are mapped in Fig. 1. B = specimens at University of British Columbia fish museum; C = reported by McCart and Pepper (1971); E = reported by Elson (1974); $E^1$ = reported by Elson (1974) but identifying characters not given; $E^2$ = reported by Elson (1974) but only found at mile 62 or below on Snake River; $E^3$ = reported by Elson (1974) but Hart and Elliott Lakes not distinguished in discussion; K = reported by Khan and Qadri (1971); M = specimens at University of Manitoba fish museum; $M^1$ = specimens at University of Manitoba fish museum, identification given in Lindsey and Franzin (1972); *65°28′N, 133°47′W; **64°39′N, 138°24′W.

| | *Stenodus leucichthys* | *Coregonus clupeaformis* | *C. autumnalis* | *Prosopium cylindraceum* | *P. coulteri* | *Thymallus arcticus* | *Salvelinus namaycush* | *S. alpinus* | *S. malma* | *Esox lucius* | *Platygobio gracilis*[a] | *Couesius plumbeus* | *Rhinichthys cataractae* | *Catostomus catostomus* | *C. commersoni* | *Lota lota* | *Cottus cognatus* | *C. ricei* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Peel River at mouth of Caribou River | | | B | | | B | | | | | B | B | | B | | B | B | B |
| 2. Mouth of Snake River | E | | E | E | | E | | E | | E | E | E | E | E | | E | E | |
| 3. Dog Lake | | | | | | | | | | | | M | | | M | | | |
| 4. Popcornfish (Crooked) Lake* | | | | B | | B | | | | | | | | B | | | | |
| 5. Snake River | | | | E | | E | | | $E^1$ | | | $E^2$ | | $E^2$ | | $E^2$ | $E^2$ | |
| 6. Margaret Lake | | EM | | | | | EM | | | EM | | | | | | | B | |
| 7. Bonnet Plume River | | | | E | | E | | | $E^1$ | | | | | | | | E | |
| 8. Fairchild Lake | | | | | | | BK | | | | | | | | | | B | |
| 9. Pinguicula Lake | | | | M | | | M | | | | | | | | | | | |
| 10. Bonnet Plume Lake | | | | EM | | EM | EM | | | | | | | | | | M | |
| 11. Hungry Lake | | | | | | | | | | EM | | | | | | | | |
| 12. Wind River | | | | E | | E | | | $E^1$ | | | | | | | | E | |
| 13. Elliott Lake | | | | EM | $M^1$ | EM | EM | $M^1$ | | | | | | EM | | | M | |
| 14. Hart Lake | | | | $E^3$ | | $E^3$ | BE | | | | | | | $E^3$ | | | | |
| 15. Ogilvie River | | | | | | M | | | | | | | | M | | | M | |
| 16. Chapman Lake | | | | M | | M | | | | | | | | M | | | | |
| 17. Blackstone River | | | | M | | BCM | | M | | | | | | | | | M | |
| 18. N. North Fork Pass Lake** | | | | M | | M | | | | | | | | | | | | |

[a] *Hybopsis gracilis* in AFS Special Publication No. 6.

NAD oxidoreductase, EC 1.1.1.8) phenotypes were determined for individual lake whitefish (*Coregonus clupeaformis* species complex) by starch-gel electrophoresis following Clayton and Franzin (1970) and Clayton et al. (1973); *ldh* allele nomenclature follows Bailey et al. (1976).

Counting of gill raker number in lake whitefish and longnose suckers and of scales along the lateral line of arctic grayling, followed the methods of Hubbs and Lagler (1958). Sculpin fin rays were counted according to McAllister and Lindsey (1961). Vertebral numbers in northern pike and lake trout were counted from radiographs; for pike, the vertebra that supports the urostyle was the last counted. For lake trout, the last upturned caudal vertebrae were counted as three, following Khan and Qadri (1971).

## Results and Discussion

At the height of Wisconsin glaciation, while the Peel was a part of the Yukon system, Bering-type fish of all species present there could have moved into the Peel basin. With the reversal of the Peel, these forms were given access to the Mackenzie delta area, and fish from the Mississippi were given access to the Peel basin. Continued gene flow of a non-Yukon racial type into the Peel basin might be expected to obscure the presence of a Yukon genetic type. In fact, there appears to have been surprisingly restricted dispersal of fish into the Peel River system from either the Bering or Mississippi refuges. Most of the fish whose origin is identifiable have come from the Bering refuge and have not been contaminated by fish from the Mississippi refuge.

### LAKE WHITEFISH (*Coregonus clupeaformis* SPECIES COMPLEX)

Lake whitefish survived Wisconsin glaciation in the Bering and Mississippi refuges (Franzin 1974); biochemical and morphological evidence indicates that distinct Yukon and Mackenzie forms of this species complex exist. The *ldh* $H\alpha^A$ allele and the G-3-PDH $B^1$ allele are totally absent from all Yukon drainage area lake whitefish populations, despite their widespread presence throughout the rest of North Amercia (Lindsey et al. 1970; Franzin 1974). Modal gill raker number shows a roughly similar pattern; gill raker

TABLE 2. Comparison of lactate dehydrogenase and glycerol-3-phosphate dehydrogenase allele frequencies and gill raker numbers for lake whitefish (*Coregonus clupeaformis*) from sites in and near the Peel River basin.

| Site | No. fish examined | Frequencies | | Gill rakers | | |
|---|---|---|---|---|---|---|
| | | *ldh* $H\alpha^A$) | (G-3-PDH $B^1$) | Mode | Mean | Range |
| Davis Lakes | 93 | 0 | 0 | 24 | 23.6 | 21–26 |
| Mackenzie Delta | 37 | 0.20 | 0.07 | 26 | 25.7 | 23–29 |
| Margaret Lake | 29 | 0 | 0 | 27 | 26.5 | 25–28 |

counts of Yukon basin populations are generally modal at 23–25 (with an occasional 26), while those of Mackenzie populations are generally modal at 26 or greater but somewhat lower in some Mackenzie delta samples (Lindsey et al. 1970).

Table 2 shows *ldh* $H\alpha^A$ and G-3-PDH $B^1$ allele frequencies and gill raker modes for Davis Lakes, Mackenzie delta, and Margaret Lake lake whitefish populations. The absence of the *ldh* $H\alpha^A$ and G-3-PDH $B^1$ alleles from Davis Lakes fish (and their typically Yukon gill raker numbers) indicates their affinity to Yukon rather than Mackenzie basin populations. This conforms to geological evidence that the Davis outlet (draining northward to the Yukon River system) must have been abandoned before the main Mackenzie basin became ice free. Hence, no Mississippi-type whitefish gained access to the Peel or Yukon while the Davis outlet operated.

Any later, post-Wisconsin, dispersal of lake whitefish into the Peel River basin would have come from a Mackenzie delta stock. The Mackenzie delta fish have strikingly lower *ldh* $H\alpha^A$ and G-3-PDH $B^1$ allele frequencies (Table 2) compared to other Mackenzie basin populations (Franzin 1974), suggesting that they are a mixture of Yukon- and Mackenzie-type stocks. In any event, both alleles are present in Mackenzie delta lake whitefish populations in sufficient frequency that if these fish had dispersed up the Peel River, the two alleles should be detectable in present Peel basin populations. The frequency of both alleles in Margaret Lake was found to be zero (Table 2). The gill raker mode is somewhat higher than in most Yukon populations perhaps due to local selection.

Clearly, Margaret Lake, which has no present surface connection to Peel waters (Bonnet Plume River), has a population of lake whitefish of pure Bering refugium origin. Furthermore, no other collections of Lake whitefish are known from anywhere in the Peel basin (McPhail and Lindsey 1970; Elson 1974). Thus, there has probably been no post-Wisconsin movement of lake whitefish up the Peel system into Yukon Territory.

## SLIMY SCULPIN (*Cottus cognatus*)

The slimy sculpin shows geographic variation in the percentage of fish having the last 2 anal rays inserting on the same basal ("last anal ray branched") as opposed to fish having the bases of the last two anal rays separate ("last anal ray unbranched") and in the percentage of pelvic fins with 3 (as opposed to 4) rays (McAllister and Lindsey 1961). In Yukon basin populations, fish with "unbranched last anal rays" are rare (0–10% of population) as are fish with only 3 pelvic rays (0–12%). Fish of the northern Mackenzie drainage and northern barren grounds are also rare with respect to "unbranched last anal rays" (0–3%), but have an intermediate frequency of 3-rayed pelvic fins (22–59%). They are perhaps the result of hybridization between Yukon-type fish and Mississippi-refuge fish, the latter having 41–100% "unbranched last anal rays" and 60–100% 3-rayed pelvic fins (McAllister and Lindsey 1961).

We have examined 219 slimy sculpins from seven sites in the Peel basin (Sites 1, 6, 8, 10, 13, 15, 17). Although some local variation exists (especially in pelvic ray counts, see McAllister and Lindsey 1961, p. 79), totals show 10.0% unbranched last anal rays and 10.4% of pelvic fins with only 3 rays. Slimy sculpins from the Bering refuge evidently populated the Peel basin during Wisconsin glaciation, and there has been no penetration of any different genetic stock into the Peel system since. Even the sculpins from farthest north on the Peel (Site 1, mouth of Caribou River, 6 specimens, 0% single last anal rays and 8% pelvic fins with 3 rays), appear to

be pure Yukon-type fish. Slimy sculpins are now known from the Mackenzie delta area (Stein et al. 1973), but their morphological affinities have not been investigated.

### Northern Pike (*Esox lucius*)

Northern pike survived Wisconsin glaciation in the Bering refugium as well as south of the continental ice; Crossman and Harington (1970) have identified dentary fragments from Late Pleistocene deposits near Old Crow. A high vertebral number form (means usually near 62) with a Bering refuge origin inhabits the Yukon basin and a lower vertebral number form (means usually near 59 or 60), which probably had a Mississippi refuge origin, is found in the Mackenzie River system (McPhail and Lindsey 1970).

Pike in Davis Lakes were found to have a mean of 62.8 vertebrae (5 specimens), while mean vertebral numbers for pike from Hungry Lake (Site 11, 3 specimens) and Margaret Lake (Site 6, 4 specimens) were 63.0 and 62.2, respectively. These relatively high counts show greater affinity to Yukon River system populations than to other Mackenzie populations.

### Arctic Grayling (*Thymallus arcticus*)

Arctic grayling lateral line scale counts show geographic variation over North America (McCart and Pepper 1971). An area of populations with low mean scale counts (below 87) exists on the North Slope and western areas of Alaska. Grayling from the Yukon River basin and British Columbia have high mean scale counts (>87), while Northwest Territories grayling are generally low with some high count populations. Eighteen fish from Cache Creek, N.W.T. (tributary to Mackenzie River at the delta) had a mean of 88.1 lateral line scales showing affinity to other Mackenzie populations. This species is known to have survived in the Bering refuge in Wisconsin times (McAllister and Harington 1969). Mean lateral line scale counts for the Peel sites of Popcornfish Lake (Site 4, 5 specimens, mean 81.4) and Ogilvie River (Site 15, 23 specimens, mean 85.0) are within the range of North Slope Alaska populations. McCart and Pepper (1971) similarly found a mean of 82.4 in fish from east Blackstone River (Site 17).

The Peel area grayling probably did not originate from Mackenzie delta grayling, which at present show no definite affinity to Peel or to North Slope Alaska fish. Peel basin grayling populations may have originated from the North Slope of Alaska, as a glacial refugium evidently did exist north of Brooks Range (as is also suggested by lake whitefish gill raker counts [Lindsey et al. 1970]). Alternatively, the Peel area might have been a separate refugium with the low scale count grayling developing in situ with little or no immigration from other areas. In the same way the pygmy whitefish (*Prosopium coulteri*), known from the Peel system only from Elliott Lake (Site 13), have distinctive characters suggesting they represent an isolated stock evolved within the refugium (Lindsey and Franzin 1972).

### Longnose Sucker (*Catostomus catostomus*)

Longnose suckers occur in two distinct morphological forms (McPhail and Lindsey 1970). A low gill-rakered type (means usually near 25 or 26) inhabits Bering Sea drainages, while a high gill-rakered type (means usually near 27 or 28) is found in Mackenzie drainage areas to the east. Longnose suckers from the Mackenzie delta area have gill raker means of about 28.

Longnose suckers from Popcornfish Lake (Site 4, 5 specimens) had a mean of 26.4 gill rakers and from Peel River at the mouth of Caribou River (Site 1, 5 specimens) a mean of 27.4. This species, judging from the small samples available, apparently has penetrated the Peel River system to some extent from the Mackenzie River area; the lower mainstream of the Peel seems to have Mackenzie-type fish, while upper Peel areas (with intermediate gill raker counts) are perhaps a mixture of Yukon and Mackenzie fish.

### Lake Trout (*Salvelinus namaycush*)

There is some morphological evidence of separation of lake trout originating from different refugia, described by Khan and Qadri (1971). Lake trout from Alaska (mean vertebral counts of 62.9), upper Yukon River basin (62.6), Liard River basin (62.3), and Peel River basin (Fairchild Lake, mean 63.2) all had similar vertebral counts significantly lower than other Mackenzie River system samples (mean vertebral counts of 64.3, 64.4, 64.6) (Khan and Qadri 1971, p. 473). We have counted lake trout vertebrae from Bonnet Plume Lake (Site 10, 9 specimens, mean 63.2), Elliott Lake (Site 13, 4 specimens, mean 62.5), and Pinguicula Lake (Site 9, 3 specimens, mean 63.3). Vertebral numbers for fish from these lakes are similar to Khan and Qadri's (1971) counts for Fairchild Lake (Site 8), and confirm that the affinity of Peel River basin lake trout lies with Yukon and Alaska fish rather than with other Mackenzie populations.

The lake trout is quite widespread in lakes of the Peel system (Table 1), but, surprisingly, has never been taken anywhere in the Porcupine

River drainage (including Davis Lakes which would seem to provide suitable habitat). The Peel system lake trout apparently had a Bering origin, even though the species is now missing from the adjacent Yukon River headwaters. Here as in several other regions the distributions of lake trout and of Arctic lamprey *Lampetra japonica* are mutually exclusive (Lindsey 1964), but whether the relationship is causal is pure conjecture.

### Trout-Perch (*Percopsis omiscomaycus*) and Lake Chub (*Couesius plumbeus*)

The trout-perch probably survived Wisconsin glaciation only in the Mississippi refuge, and only recently has invaded the Yukon River system (McPhail and Lindsey 1970). This is evidenced by its Yukon basin distribution which is largely restricted to the Yukon mainstem, especially towards the river mouth, and by the apparent lack of distinct Yukon and Mackenzie forms. The lake chub has a similar distribution to the trout-perch but is more widespread in Yukon drainages, and so may have invaded the Yukon basin somewhat earlier.

If these two species gained access to the Yukon basin via the Peel when it discharged through the Davis outflow channel, they should now be present in the Peel. No trout-perch have been recorded from anywhere in the Peel system upstream of the Northwest Territories boundary. Lake chub are found in the Peel River system only as far upstream as mile 62 of the Snake River, and in Dog Lake (Site 3). The presence of white suckers *Catostomus commersoni* in Dog Lake but nowhere farther up the Peel system nor in the Yukon River system suggests that the lake has been entered via its outlet, by lake chub and white suckers, since the last ice retreat. Thus, lake chub and trout-perch probably did not reach the Yukon River via the Peel. These two, and perhaps other species, may have entered the Yukon system via Summit Lake at McDougall Pass in post-Wisconsin times during periods of high water when the Mackenzie and Yukon Waters may be confluent.

### Concluding Remarks

Zoogeographic conclusions based on single morphological characters, in several of the species discussed, have obvious limitations. In all cases, only characters that differ significantly between undoubted Bering and Mississippi populations and whose pattern of variation does not seem to be aligned with climatic gradients have been used. Using these characters, six of the forms in Peel basin seem to be either of Bering origin or have developed in situ (lake whitefish, slimy sculpin, northern pike, Arctic grayling, pygmy whitefish, lake trout).

It is surprising that there has not been more dispersal of fish into the Peel River system, during and after the Wisconsin. While the Peel formed a part of the Yukon River system, two coregonids, *Coregonus nasus* and *C. sardinella* (both of which now occur in Davis Lakes), and three *Oncorhynchus* species presumably had access to the Peel system but did not become established there. In post-Wisconsin times, no less than 18 species of fishes have had access to Peel waters from the Mackenzie delta area and yet have penetrated poorly or not at all up the Peel River system (Table 1; McPhail and Lindsey 1970; Stein et al. 1973; Jessop and Lilley 1975). Furthermore, only six or seven fish species could be considered widespread in the Peel basin (Table 1).

No single waterfall or rapid is known on the Peel River which could act as an obstruction to the ascent of fish, although the river runs swiftly over its whole course with deeply incised banks, turbid water, and shifting bottom. The feature distinguishing it ecologically from the Mackenzie (which is much richer in fish species) may be temperature. The Mackenzie rises far to the south and carries an immense body of relatively warm water to the Arctic Ocean, along with a tongue of warmer microclimate and accompanying plants and animals. The Peel, in contrast, drains a cold northern region of sparsely vegetated plateaux and mountains. The scarcity of lakes (characteristic of unglaciated terrain) may also contribute to the unsuitability of the area to many fish species. Presumably during the ice advances the waters of the ice-dammed lakes were cold, silt-laden, and unproductive. The paucity of fish species inhabiting the Peel basin is probably due more to the present physical environment of the area than to a lack of opportunity for access.

Several other fish species not discussed in detail may have used the Peel River reversals to gain access from the Yukon to the Mackenzie rivers or vice versa. Often it is difficult to disentangle this route from alternatives such as a Liard–upper Yukon route, or brackish water dispersal along the arctic coast.

The salient feature of Peel basin fishes seems to be that, although this is a Mackenzie River tributary, many of its populations represent relict pockets of Yukon River origin. Without knowledge of the great drainage reversals brought on by glaciation, the zoogeography of the area would be incomprehensible; with it, the biological and geological data are mutually reinforcing.

The persistence of these distinctive stocks demonstrates that here is an extensive unglaciated refugium unique in Canada. The region probably supports many other distinctive forms of life, particularly invertebrates and plants, with highly restricted distributions. The aquatic forms may be, like the Margaret Lake whitefish, confined to one or a few water bodies; they are particularly vulnerable to man's activities.

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# Filamentous Algae as a Food Source for the Brown Bullhead (*Ictalurus nebulosus*)[1]

J. M. GUNN, S. U. QADRI

*Department of Biology, University of Ottawa, Ottawa, Ont. K1N 6N5*

AND D. C. MORTIMER

*Division of Biological Sciences, National Research Council of Canada, Ottawa, Ont. K1A 0R6*

GUNN, J. M., S. U. QADRI, AND D. C. MORTIMER. 1977. Filamentous algae as a food source for the brown bullhead (*Ictalurus nebulosus*). J. Fish. Res. Board Can. 34: 396–401.

Studies of food habits of the brown bullhead (*Ictalurus nebulosus*) in the Ottawa River have often shown a high proportion of filamentous algae in the stomach. This study was designed to determine whether the algae were utilized as food. Isotopic and caloric techniques showed that about 23% of the carbon from *Spirogyra* and 67% of the carbon from *Anaebena flos-aquae* was assimilated by the fish within 24 h of ingestion.

GUNN, J. M., S. U. QADRI, AND D. C. MORTIMER. 1977. Filamentous algae as a food source for the brown bullhead (*Ictalurus nebulosus*). J. Fish. Res. Board Can. 34: 396–401.

On a souvent observé, lors d'études sur les habitudes alimentaires de la barbotte brune (*Ictalurus nebulosus*) de la rivière des Outaouais, une forte proportion d'algues filamenteuses dans l'estomac. La présente étude a été entreprise en vue de déterminer si les algues étaient utilisées comme nourriture. Des techniques isotopiques et caloriques démontrent qu'environ 23% du carbone de *Spirogyra* et 67% du carbone d'*Anaebena flos-aquae* sont assimilés par le poisson en dedans de 24 h après ingestion.

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STOMACH analyses have demonstrated that brown bullhead (*Ictalurus nebulosus*) ingest large quantities of filamentous algae (Cable 1929; Rubec 1975). Although several fish species are known to consume algae, general uncertainty has remained as to its digestibility (Gerking 1962; Rabe et al. 1973; Klarberg and Benson 1975).

In the Ottawa River algae often represented more than 60% of the bullhead diet (Gunn 1976). This large quantity included a variety of species: *Spirogyra* sp., *Ulothrix* sp., *Oscillatoria* sp., and *Fragilaria* sp. Reported here are experiments to determine whether a significant fraction of the carbon from two of these species, *Spirogyra* sp. and *Anabaena flos-aquae*, was assimilated by the brown bullhead.

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## Materials and Methods

Digestion of the algae by the brown bullhead gut was demonstrated quantitatively by radioactive labeling ($^{14}C$), by mass loss, and by caloric content changes. Visual evidence was obtained from photomicrographs of algae before and after digestion.

Brown bullhead (90–200 g), netted in the Ottawa River, were placed in separate 50-$\ell$ aquaria containing about 40 $\ell$ of aerated river water maintained at 20 ± 2°C.

During the 8 wks before each experiment, the fish were trained to accept minnows and trout chow in gelatin capsules (no. 0) by hand feeding. The experiment was started by substituting a capsule loaded with $^{14}C$-labeled algae.

The *Spirogyra* sp. was collected from the Ottawa River, thoroughly washed, and placed in a $^{14}C$-labeled medium ($NaH^{14}CO_3$ in tap water). After photosynthesizing under strong sunlight for 3 h, the algae were rinsed and allowed to grow in fresh water for 3 additional days. Algae were then harvested, washed, blotted dry, placed in gelatin capsules, and fed to the fish.

*Anabaena flos-aquae* (No. 1444) obtained from the Indiana University culture collection (Starr 1964), was grown in culture medium ASM-1 of McLachlan and Gorham (1961). The medium was prepared in 8-$\ell$ bottles and autoclaved before inoculation. The

cultures were aerated and maintained under continuous light at 20°C. *Anabaena flos-aquae* grew rapidly under these conditions and required harvesting 8 days after inoculation. 100 $\mu$Ci $NaH^{14}CO_3$ was added to each bottle 2 days prior to harvesting. The algae were harvested by centrifugation and resuspended in unlabeled medium. After the final centrifugation, four culture flasks (50 ml) of the sterilized medium were inoculated to demonstrate the viability of the algae.

### Digestion of $^{14}$C-Labeled Algae

The intent of the experiment using *Spirogyra* sp. was to demonstrate active digestion by measuring tissue concentrations of $^{14}$C and to determine efficiency of carbon assimilation from ingested algae. The samples of labelled *Spirogyra* sp. (0.574–0.916 g wet wt) were packed in gelatin capsules as live intact filaments and fed to the fish. Two fish were killed at each of the 9-, 12-, and 24-h intervals after ingestion. From each fish various tissues were removed, weighed, and frozen for analysis of $^{14}$C content. The alimentary tract was carefully opened and washed free of feces. All feces and washings were collected and frozen. In this experiment the total recovered count (counts per minute $^{14}$C tissue + feces + washings) was used as a measure of the original ingested counts.

Modifications were used in the *A. flos-aquae* experiment to more accurately measure ingestion by carefully weighing the algae (0.2270–0.3261 g wet wt) into the capsules. The $^{14}$C content was estimated from a conversion factor established by accurately counting the $^{14}$C in separate subsamples (0.6266–1.1021 g wet wt) of the same algal mass.

Water samples (50 ml) were collected at 6-h intervals. Particulate matter (regurgitated algae, feces) was collected on Millipore filters (0.45 $\mu$m) and counted. Carbon dioxide in the filtrate was precipitated as $BaCO_3$, collected on Millipore filters (0.45 $\mu$m) (Sorokin 1968) and counted for $^{14}$C content. Aliquots of the second filtrate were counted to detect $^{14}$C-labeled soluble metabolic products.

Twenty-four hours after ingestion the fish were killed. The 24-h duration was selected after preliminary experiments demonstrated an evacuation time of approximately 30 h at the experimental temperature (20°C). After 24 h the fecal material was in the hindgut section where it could be easily collected. That relatively little absorption occurs in the hindgut (Neff and Musacchia 1967) was confirmed in the *Spirogyra* experiment.

After killing the fish, the feces was washed from the alimentary tract and collected. The aquarium water was given a brisk stirring and the final samples (2 × 400 ml) siphoned off. The counts of particulate matter, respired $^{14}CO_2$, and soluble excretions were determined as indicated above. Totals were based on the measured volume of the aquarium water.

### Tissue Preparation and Counting Procedure

Algae were dried (65°C) to constant weight. The dried algae were then ground in a glass homogenizer and suspended in distilled water (150 ml). The suspension was maintained by a magnetic stirrer and subsamples (1 ml) taken for dry weight and counting.

The individual tissues (e.g. liver, spleen) were prepared and counted separately in the *Spirogyra* sp. experiment. Muscular tissues (e.g. stomach, lateral muscle) were finely sliced before homogenization. The carcass remains (74.06–137.71 g wet wt) were first macerated (Sorvall omnimixer) and then subsamples (5.97–11.05 g wet wt) taken for final homogenation. Tissues were homogenized in a measured volume of water at high speed (Sorvall 1500 rpm).

In the *A. flos-aquae* experiment the alimentary tract was the only tissue removed and counted separately. The carcass remains were subsampled as described above and the same homogenizing procedure used. Similar emulsions were prepared for the fecal samples.

Duplicate 1-ml samples of each tissue were counted for 1 min in the Beckman LS200 liquid scintillation counter (Amersham-Searle PSC solubilizer was the counting "cocktail"). All samples were quench corrected with an internal standard ($^{14}$C-labeled toluene). The base line of the $^{14}$C-window was raised above the peak of fish tissue fluorescence. Background corrections were applied to all samples.

### Mass Balance Assimilation Efficiency

The mass balance experiment with *A. flos-aquae* was conducted parallel to the labeled carbon experiment. For both experiments the same feeding procedure (algae in capsules), number of fish (4), and experiment duration (24 h) were used. The experiments differed in that the mass balance experiment used unlabeled algae and the assimilation efficiency was measured in terms of mass and caloric changes of the ingested algae.

The algae in capsules (0.5164–0.5950 g wet wt) and analytical samples (1.4841–3.4286 g wet wt) from the same algae mass were carefully weighed before feeding the fish. The fish were killed after 24 h and feces collected from the alimentary tract. The algae and fecal samples were dried (65°C) to constant weight. Pellets of the sample material were pressed, weighed (15–22 mg), and caloric value determined in the Phillipson microbomb calorimeter. No corrections for intestinal mucus or capsule remains in the feces were applied.

### Morphological Changes of Digested Algae

Photomicrographs were taken of *A. flos-aquae* (×563) and *Spirogyra* sp. (×90) under phase-contrast using a Zeiss microscope. The *Spirogyra* sp. (No. 1343) used for these pictures was obtained from the Indiana University culture collection. Photomicrographs were taken of the algae before ingestion and then again 24 h later. The material for the post-digestion pictures was obtained from the hindgut section of the alimentary tract.

TABLE 1. Relative tissue concentration $\frac{\text{cpm/g tissue}}{\text{cpm/g total fish}}$ of $^{14}C$ in brown bullhead (*Ictalurus nebulosus*) following ingestion of $^{14}C$-labeled *Spirogyra* sp.

| | Time after ingestion | | | | | |
|---|---|---|---|---|---|---|
| | 9 h | | 12 h | | 24 h | |
| Fish no. | 1 | 2[a] | 3 | 4 | 5 | 6 |
| Tissues | | | | | | |
| Liver | 3.04 | 1.98 | 3.22 | 3.60 | 3.11 | 4.99 |
| Spleen | 1.00 | 1.37 | 2.14 | 1.65 | 2.19 | 2.73 |
| Kidney | 2.92 | 1.29 | 2.81 | 2.96 | 6.34 | 7.06 |
| Gonad | 1.24 | .59 | 1.49 | 1.98 | 1.45 | 1.19 |
| Stomach | 2.15 | 1.36 | 2.41 | 1.77 | 8.23 | 2.35 |
| Foregut | 43.22 | 15.92 | 29.02 | 39.92 | 27.84 | 22.51 |
| Midgut | 6.04 | 1.62 | 4.83 | 10.91 | 5.18 | 14.09 |
| Hindgut | 1.68 | .45 | 1.33 | 2.34 | 4.01 | 5.29 |
| Lat. muscle | .42 | .22 | .32 | .61 | .53 | .42 |
| Remaining carcass | .54 | .82 | .56 | .57 | .61 | .51 |

[a]Values for fish 2 were presumably affected by contamination of body tissues with feces from the alimentary tract.

TABLE 2. Total recovery of $^{14}C$ and percent recovered in brown bullhead tissues following ingestion of $^{14}C$-labeled *Spirogyra* sp.

| | Time after ingestion | | | | | |
|---|---|---|---|---|---|---|
| | 9 h | | 12 h | | 24 h | |
| Fish no. | 1 | 2[a] | 3 | 4 | 5 | 6 |
| $^{14}C$ cpm ($\times 10^6$) recovered in: | | | | | | |
| Fish tissue | 4.4 | 4.7 | 4.7 | 5.0 | 5.5 | 4.1 |
| Feces and washings | 15.6 | 8.2 | 20.2 | 13.7 | 14.7 | (egested) |
| Total | 20.0 | 12.9 | 24.9 | 18.7 | 20.2 | |
| % of total $^{14}C$ recovered in tissues | 21.9 | 36.6 | 19.0 | 26.7 | 27.2 | |

[a]Values for fish 2 were presumably affected by contamination of body tissues with feces from the alimentary tract.

## Results

The $^{14}C$ concentrations in the several tissues of individual fish form a consistent pattern (Table 1). The highest concentration of $^{14}C$ was initially in the foregut, but levels in this tissue progressively decreased during the 24-h interval. Digestion products were transported through the circulatory system and marked accumulations of labeled products occurred in the liver, spleen, and kidney.

Defining assimilation efficiency as the proportion of $^{14}C$ recovered in fish tissue to the total $^{14}C$ recovered, the *Spirogyra* sp. was assimilated at an overall efficiency of $23.7 \pm 3.2\%$, excluding the data for the aberrant fish (Table 2). Digestion was essentially complete after only 9 h.

For this estimate of assimilation efficiency the respiratory and excretory losses were considered minimal (except fish 6). The tissue counts and the total recovered counts were used as measures of the assimilated and the ingested counts, respectively. In the *A. flos-aquae* experiment the $^{14}C$ content of the algae contained in the capsule was measured at the time of feeding, and the fate of the ingested algal carbon was determined by measuring $^{14}C$ in the various compartments.

The results from the *A. flos-aquae* experiment

TABLE 3. Brown bullhead assimilation of algal carbon (as $^{14}C$ counts per minute) 24 h after ingesting $^{14}C$-labeled *Anabaena flos-aquae*

| | | $10^6$ cpm[a] | % |
|---|---|---|---|
| Assimilated $^{14}C$ | Carcass | 2.65±0.20 | 76.8±0.9 |
| | Gut | 0.80±0.02 | 20.9±1.2 |
| | $^{14}C$ $CO_2$ in aquarium water | 0.08±0.01 | 2.3±0.3 |
| | Total cpm | 3.45±0.27 | 100 |
| Unassimilated $^{14}C$ | Feces from GI tract | 1.69±0.13 | 77.9±0.4 |
| | Water particulates | 0.06±0.04 | 3.3±2.0 |
| | Soluble excretions | 0.33±0.06 | 18.8±1.9 |
| | Total cpm | 2.08±0.17 | 100 |
| Total ingested counts | | 6.92±0.27 × $10^6$ cpm | |
| Total recovered counts | | 5.52±0.12 × $10^6$ cpm | (80.3±3.7%) |
| Unaccounted for $^{14}C$ (presumed lost as $^{14}CO_2$) | | 1.39±0.31 × $10^6$ cpm | (19.7±3.7%) |
| Assimilation efficiency: | Assimilated $^{14}C$/Ingested $^{14}C$ | | 48.1±5.6% |
| | Assimilated $^{14}C$/Ingested $^{14}C$ plus lost $^{14}CO_2$ | | 67.5±3.6% |

[a]Means and SD for data for four similarly treated fish.

TABLE 4. Brown bullhead assimilation of *A. flos-aquae* 24 h after ingestion; by caloric and gravimetric methods[a]

| | Caloric value (*J/g*) | Dry weight (*g*) | Caloric content (*J*) |
|---|---|---|---|
| Ingested algae | 18113 | 0.1207±0.0041 | 2186±74 |
| Feces (from hindgut) | 13910±610 | 0.0660±0.0016 | 917±47 |
| Loss | 4203 | 0.0547 | 1269 |
| Assimilation efficiency[b]: | Gravimetric | 45.3±1.1% | |
| | Caloric | | 58.0±2.2% |

[a]Averages of values for four similarly treated fish.
[b]Assimilation efficiency was determined from the weight and caloric losses expressed as percentages of the original values.

show that this algae is utilized more effectively than the *Spirogyra* sp. (Table 3). Although the specific activity of the *Anabaena* was constant for all 4 fish (2.74 × $10^7$ cpm/g fresh weight), the different weights in the capsules accounted for the range of ingested counts around 6.92 × $10^6$ cpm. This obviously contributed to variations in the total assimilation but the scatter in the data was small and the percent of total in each fraction was again remarkably consistent between fish. The assimilation efficiency of the blue-green *A. flos-aquae* averaged 67% if the missing $^{14}C$ is counted as lost $^{14}CO_2$, or 48% if only the recovered counts are included.

The amount of $^{14}CO_2$ respired by the fish and lost to the atmosphere during the 24-h experiment was not established. It is certain that $^{14}CO_2$ was in the water after only 6 h and that after 24 h the analyzed concentration in the aquarium water accounted for only 2.3% of the ingested total. Since the aquaria were continuously aerated, it is reasonable to assume that the 19.7% of ingested carbon which was not accounted for was lost through aerated gas exchange.

The assimilation efficiencies of the algae determined by gravimetric and caloric methods were 45.3 and 58.0%, respectively (Table 4). The ingested algae had a moisture content of 78.1% and a caloric value of 18,113 J/g. This was reduced to 13,910 J/g after 24 h with 54.7% of the initial weight remaining.

The analytical data for this sample of *A. flos-*

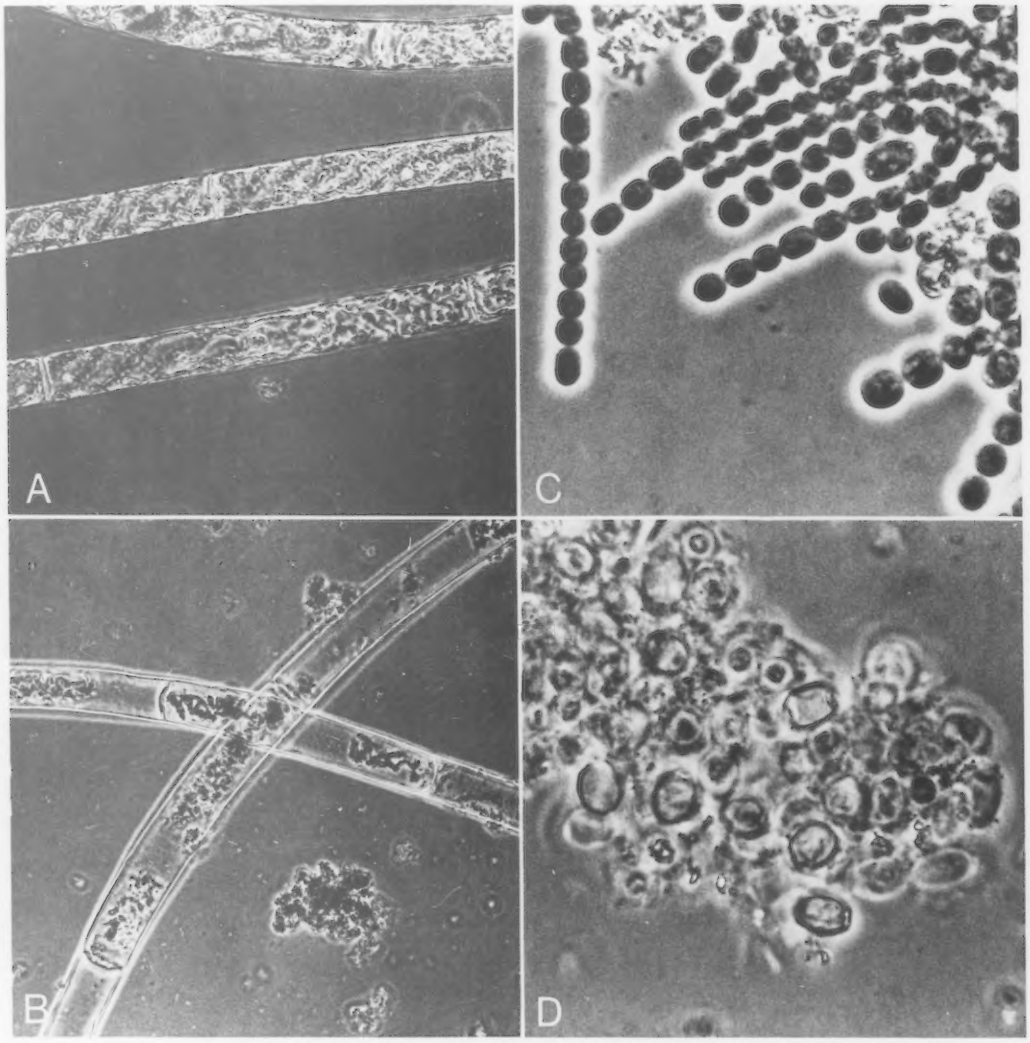


FIG. 1. Photomicrographs of filamentous algae ingested by brown bullhead (*Ictalurus nebulosus*). A, *Spirogyra* sp. before ingestion (×90); B, *Spirogyra* sp. 24 h after ingestion (×90); C, *Anabaena flos-aquae* before ingestion (×563); D, *Anabaena flos-aquae* 24 h after ingestion (×563).

*aquae* are:

| % carbon | % hydrogen | % ash | % nitrogen | % crude protein |
|---|---|---|---|---|
| 42.1 | 6.1 | 14.9 | 7.6 | 47.6 |

Although the ash is relatively high, conversion of the Table 4 values to an ash free basis does not significantly alter the assimilation efficiency.

## MORPHOLOGICAL CHANGES IN INGESTED ALGAE

*Spirogyra* sp. maintained its filamentous form 24 h after ingestion, although most of the cells were fractured and void of most of their contents (Fig. 1A, B). The few intact cells recovered were green and grew when placed in fresh water. The presence of viable cells in feces samples suggested

to some authors that the algae was indigestible (Rabe et al. 1973).

For *A. flos-aquae* under the same conditions, the color and morphological changes were striking. The original blue-green color was changed to dark brown, and filaments were disintegrated with only clusters of heterocysts remaining (Fig. 1C, D). All cells that did remain in filamentous form were plasmolyzed.

## Discussion

The special attention directed toward reducing stress factors that could adversely affect digestion (Fish 1960; Moriarty 1973) and the use of fresh, actively growing algae of the type recovered from Ottawa River fish produced experimental data that should be directly representative of the natural habits of the fish.

Although the digestibility data were remarkably consistent between fish and between methods of assessment, the values reported are probably low. Several factors that would tend to reduce digestion include: slow disintegration of the capsule (1 h), absence of the usual sand in the stomach, and the isolated, inactive environment of the experimental fish (Ivlev 1961).

That both species of algae are extensively metabolized is evident from the recovery of $^{14}C$-labeled compounds from all organs and tissues 9 h after ingestion (Table 1, *Spirogyra*) and $^{14}CO_2$ in the water after only 6 h. The difference in digestibility between *Spirogyra* and *Anabaena* correlates with their respective crude protein contents of 22 and 48% (Boyd 1968, 1973). Difference in digestibility between algal species was noted in other studies with protozoan and microbiological organisms (Arnold 1971; Infante 1973; Gunnison and Alexander 1975).

Because brown bullhead from the Ottawa River have at some seasons a high proportion of filamentous algae in their stomach contents and because this type of algae was clearly shown to be metabolized by bullhead, algae must be regarded as a significant food source for this species.

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# Isolation and Characterization of an Anaerobic Bacterium, *Eubacterium tarantellus* sp.nov., Associated with Striped Mullet (*Mugil cephalus*) Mortality in Biscayne Bay, Florida

LANNY R. UDEY, ELAINE YOUNG, AND BENNETT SALLMAN

*Department of Microbiology, University of Miami School of Medicine, Miami, Fla. 33152, USA*

UDEY, L. R., E. YOUNG, AND B. SALLMAN. 1977. Isolation and characterization of an anaerobic bacterium, *Eubacterium tarantellus* sp.nov., associated with striped mullet (*Mugil cephalus*) mortality in Biscayne Bay, Florida. J. Fish. Res. Board Can. 34: 402–409.

An asporogenous, gram-positive, anaerobic bacterium was isolated in pure culture from the brains of numerous dead and moribund striped mullet (*Mugil cephalus*) from Biscayne Bay, Florida. Biochemical characterization confirmed that it was a new species and has been named *Eubacterium tarantellus* (ATCC 29255). All isolates produced lecithinase and deoxyribonuclease, were beta hemolytic, but only weakly fermented selected carbohydrates. This anaerobe was pathogenic for channel catfish (*Ictalurus punctatus*) but not for white mice or guinea pigs.

More recently *E. tarantellus* has been isolated from 10 additional fish species. This and other anaerobic bacteria may be significant pathogens of estuarine fish.

*Key words*: Anaerobic bacteria, *Eubacterium*, *Mugil cephalus*, and fish diseases.

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Nous avons isolé une bactérie anaérobie, Gram-positif et asporogène dans une culture pure de cerveaux de plusieurs muges cabots (*Mugil cephalus*) morts et moribonds de la baie Biscayne, Floride. Ses caractéristiques biochimiques confirment qu'il s'agit d'une espèce nouvelle, à laquelle on a donné le nom de *Eubacterium tarantellus* (ATCC 29255). Tous les isolats produisent de la lécithinase et de la désoxyribonucléase, sont bêta-hémolytiques, mais ne produisent qu'une faible fermentation d'hydrates de carbone choisis. Cette bactérie anaérobie est pathogéne pour la barbue de rivière (*Ictalurus punctatus*), mais ne l'est pas pour les souris blanches ou les cobayes.

*E. tarantellus* a été isolé tout dernièrement de 10 autres espèces de poissons. Ce microbe, ainsi que d'autres bactéries anaérobies peuvent être des agents pathogènes importants de poissons d'estuaires.

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BACTERIA known to be pathogenic to fish are characteristically either aerobic or facultatively anaerobic organisms. Although obligately anaerobic bacteria can be isolated from aquarium water containing ornamental fish (Trust and Bartlett 1974) and from terrestrial and marine environments (Dowell 1975) they have never been shown to cause disease in fish.

In recent years there has been an upsurge of interest in anaerobic bacteria and their role in diseases of man and animals (Sutter and Finegold 1973). Anaerobes have been implicated in no less than 25 different types of infections in man. Even though it is well known that anerobes are significantly involved in infections they remain the most frequently overlooked disease agents.

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The detection of these microorganisms is complicated by the frequent contamination of samples by faster growing facultative bacteria (Gorbach and Bartlett 1974). They are also oxygen sensitive, being readily killed by exposure to air, and many are nutritionally fastidious. Until adequate clinical anaerobic laboratory facilities and techniques were used, up to 50% of human abscesses were reported to contain "sterile fluid" (Gorbach and Bartlett 1974). More careful examination of these "sterile abscesses" often revealed that they were colonized by fastidious anaerobic microorganisms.

Although some anaerobic infections are due to exogenous organisms (e.g. *Clostridium tetani* wound infections), most infections are due to organisms from endogenous sources. In man, the most frequent sources of endogenous bacteria are the intestine, mouth, and genital tract (Sutter and Finegold 1973). *Bacteroides*, *Clostridium*, and

*Propionibacterium* are the three most commonly implicated genera in anaerobic infections; however, other anaerobes can be major pathogens. Members of the genus *Eubacterium* have been found to cause soft tissue abscesses in humans (Smith 1975).

In a recent communication (Udey et al. 1976) we reported on a gram-positive anaerobic rod that can produce neurologic disease symptoms (twirling) and death in striped mullet (*Mugil cephalus*). The organism was discovered as a result of an investigation into the cause of several major fish kills in Biscayne Bay (Miami, Florida). This anaerobe was first identified only as a member of the genus *Eubacterium* but is further described here as a new species, *E. tarantellus*.

## Materials and Methods

Dead, moribund, and "normal" striped mullet were obtained by net from the saltwater basins of two south Florida tributary canals of Biscayne Bay. All fish were either placed on ice immediately after capture or transported alive to the laboratory. Gross pathology was recorded for all fish.

Brain tissue was fixed in Formalin–ammonium bromide solution. Tissues were dehydrated and cleared using tetrahydrofuran and embedded in paraffin, according to American Registry of Pathology (Luna 1968), Method IV. Ten-micrometer-thick sections were cut and stained using the Brown and Brenn (1931) modified Gram stain.

Initially, vital organs were aseptically removed and cultured for aerobic microorganisms on brain heart infusion agar (BHIA, Difco). Tissues were additionally cultured in Brewer's thioglycollate medium (BTM, Difco) for detection of anaerobic microorganisms as outlined by the Center for Disease Control (Dowell and Hawkins 1974). All cultures were incubated at room temperature (RT) for at least 7 days to detect slow-growing organisms.

Cultures of brain tissue that gave rise to obligate anaerobes were cloned on BHIA and incubated at RT for 48 h in a Gas-Pak (BBL) incubator. Well-separated, rhizoid colonies were subcultured and maintained in BTM. Representative cultures (79, 82, 84, 86, and 87) from five fish were used for this study. Strain 87 was chosen as the type strain, submitted to the American Type Culture Collection, and carries the accession number, ATCC 29255.

Cell morphology and motility were determined by phase-contrast microscopy of overnight cultures incubated in BTM, and representative cells were photographed. Colony appearance was described after 48–72 h anaerobic incubation on BHIA.

Ultrastructure was determined by growing strain 79 in brain heart infusion (BHI, Difco) anaerobically for 24 h at RT. The culture was harvested by centrifugation, washed once in Millonig's buffer (Millonig 1961), and resuspended in 2.5% glutaraldehyde fixative. Fixed cells were washed in Millonig's buffer and post-fixed in Dolton's chrome osmium (Dolton 1955) at 4°C for 18 h and again washed in Millonig's buffer. The resultant pellet was dehydrated in an ethanol series of 25, 50, and 75%, each for 45 min. During the 75% dehydration cells were stained with uranyl acetate (Gibbons and Gristone 1960). Cells were further dehydrated in 100% ethanol and in three changes of acetone. Pellets were incubated for 2 h in 1:1 acetone–epon, 1 h in 1:4 acetone–epon, 1 h in epon, and embedded in epon overnight at 60°C (Luft 1961). Silver-grey sections were cut on an LKB, type 4802A ultramicrotome, using an IVIC diamond knife and mounted on carbon-coated formvar grids. These sections were then counterstained with Reynolds' lead citrate (Reynolds 1963). Electron micrographs were taken on a Jeolco, Model JEM-7 electron microscope.

Carbohydrate fermentations and assays for gelatin hydrolysis, indole production, milk reaction, nitrate reduction, lecithinase, lipase, and catalase were done in accordance with procedures outlined by Dowell and Hawkins (1974). Results were recorded at 2 and 7 days. In addition, the five chosen cultures were sent to the Anaerobe Laboratory at CDC for confirmation of most tests.

The drug sensitivity of these anaerobes was determined by the procedures supplied with Bacto Sensitivity Discs (Difco). Additionally, the organism was grown in BHI containing gentamicin (Schering) at a concentration up to 100 $\mu$g/ml, or 7.5 $\mu$g/ml vancomycin (Lilly), or 100 $\mu$g/ml neomycin (Sigma), or 300 U/ml polymyxin B sulfate (Sigma).

The ability of the anaerobe to grow at 4–44°C as well as to grow or survive in 0.5–10% NaCl was assayed in BTM. Brain heart infusion, adjusted to pH's from 3 to 9, was used to determine the pH range for growth and survival.

Sheep blood in BHIA base was used to determine hemolysin activity. Production of deoxyribonuclease was determined after streaking the organism on pre-reduced DNAase test agar (BBL) and incubating for 48 h.

The method of Holdeman and Moore (1975) was followed to detect fermentation by-products. A Dorhmann "Anaerobic Bacteriology (Anabac) Analyser" GLC with thermal conductivity detector was the instrument used in these analyses.

Samples for analysis were taken from 48 h cultures grown in PRAS–peptone–yeast–glucose medium (PY–glucose; Scott Laboratories).

To establish whether the microorganism produces toxins, filter-sterilized (Millipore, 0.22 $\mu$m) supernatant from a 48-h chopped meat medium (CMM) culture was injected into 30-g white mice. Each of five mice was injected intraperitoneally (ip) with 0.1 ml of serial tenfold dilutions of the supernatant. Control animals were injected with uninoculated CMM supernatant. All mice were observed for 2 wk.

Pathogenicity of the organism for mammals was determined by the standard test for guinea pig virulence (Dowell and Hawkins 1974). The organism was grown in BHI with 0.03% thioglycollic acid (Difco) overnight, washed, and resuspended in fresh

BHI to remove the thioglycollic acid. Six guinea pigs were used. Two were given a 1.0-ml intramuscular (im) injection of the washed culture mixed 1:1 with sterile 10% $CaCl_2$ and two were given a 1.0-ml im injection of the washed culture mixed 1:1 with sterile 0.85% saline. Saline and $CaCl_2$ control animals were injected in the same manner, using sterile BHI medium in place of the washed culture. A portion of each inoculum was cultured in BTM to confirm viability. The animals were observed for evidence of pathology for 20 days.

Six channel catfish (*Ictalurus punctatus*), ranging in size from 7.5 to 13.0 cm were injected ip with 0.1 ml of a BTM culture. Control fish were similarly injected with sterile BTM and all fish observed daily for 1 mo. Fish that died were necropsied and brain tissue was placed in BTM. At the end of 1 mo, all surviving fish were sacrificed, necropsied, and their tissues cultured for the presence of the anaerobe.

## Results

The fish caught alive showed little or no external pathology. Necropsy revealed a low number of trematodes (*Bucephalus* sp.) in occasional fish. Approximately 20% of the fish had low numbers of *Myxosoma cephalus* spores in the brain cavity but little other internal pathology was observed.

Analyses of liver and brain tissue revealed no abnormal levels of chlorinated hydrocarbon pesticides.

The behavior of the fish implied neurological impairment and suggested a central nervous system associated condition. For this reason, brain samples were prepared for histopathology. Brown and Brenn stained sections showed the presence of a large gram-positive rod throughout the brain tissue (Fig. 1). These rods displayed a tendency to form long unbranched filaments.

Tubes of BTM inoculated with brain tissue produced growth of an organism in apparently pure culture. This growth was evident after overnight incubation at RT and was confined to the reduced portion of the tube. Subculture to aerobically incubated BHIA plates revealed that the organism would not grow in the presence of oxygen.

Occasional vibrios, biochemically resembling but serologically distinct from *Vibrio anguillarum*, were isolated from kidney and liver on aerobically incubated plates. No bacteria appeared on aerobically incubated plates inoculated with brain tissue.

Colonies of the anaerobe on BHIA were flat, translucent, colorless, and rhizoid, with a distinct "pinwheel" appearance. They ranged in size from 2 to 5 mm and were soft and slightly mucoid. A large zone of beta hemolysis was present around colonies on sheep blood agar plates. In BTM the organism grew as discrete clusters which were fluffy and filamentous. The clusters of growth were often surrounded by a slimy layer.

When initially isolated in BTM, the organism had the same tendency to form long unbranched filaments (Fig. 2) as seen in brain tissue. The filaments readily broke up into individual rods after several transfers. These rods were large (1.3–1.6 × 10.0–17.0 $\mu$m), nonmotile, and gram positive. Spores were not observed by phase-contrast microscopy in cells grown in CMM. Heat-resistant spores could not be demonstrated by the method of Holdeman and Moore (1975). The cell ultrastructure (Fig. 3) is typical of many gram-positive bacteria. The cell wall is a single layer and the cytoplasm is uniformly dense and regular. A typical mesosome can also be seen.

All strains grew well between 25 and 37°C. Growth at 15 or 40°C was marginal and though the organism survived for at least 2 wk at 4°C, it did not grow at this temperature. The organism could be recovered after incubation at 42°C for 24 h but did not grow at that temperature. It did not survive 44°C for 24 h.

All isolates grew well in BTM maintenance medium and in prereduced BHI. The addition of 0.03% thioglycollic acid enhanced growth in BHI.

The organism grew in BTM with added NaCl up to a concentration of 2%, but could be recovered after 24 h incubation in concentrations up to 10%.

All cultures grew within 72 h in BHI adjusted to an initial pH from 5.6 to 8.0. The organism did not grow at pH extremes outside this range; however, it did survive for 24 h at pH 3.4 and 8.8 (Table 1).

To develop a selective medium for isolation from environmental samples, the antibiotic sensitivity of these isolates was studied. Antibiotic susceptibility of *E. tarantellus* from striped mullet, where S = sensitive, I = intermediate sensitivity, and R = resistant, is as follows:

| Antibiotic | Susceptibility (five strains) |
|---|---|
| Erythromycin | S |
| Chloramphenicol | S |
| Penicillin | S |
| Tetracycline | S |
| Novobiocin | S |
| Vancomycin | S |
| Streptomycin | I |
| Neomycin | R |
| Kanamycin | R |
| Polymyxin B | R |
| Gentamicin | R |

All cultures were sensitive to erythromycin, chlor-

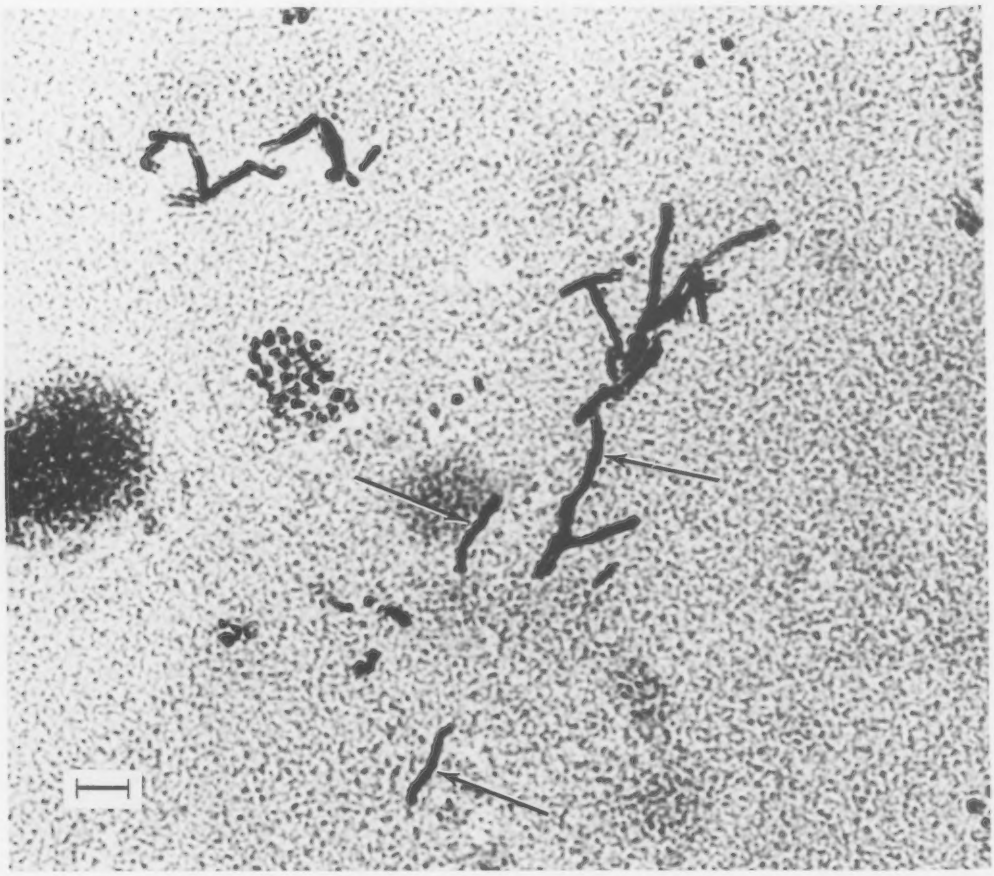

FIG. 1. Brown and Brenn stained section of striped mullet (*Mugil cephalus*) brain tissue showing large gram-positive rods (arrows). Bar = 10.0 μm.

amphenicol, penicillin, tetracycline, and novobiocin as determined by zones of inhibition surrounding the discs. The organism was resistant to kanamycin, while sensitivity to streptomycin was of the intermediate range.

All strains grew well in BHI containing 100 μg/ml gentamicin, 100 μg/ml neomycin, or 300 U/ml polymixin B. Vancomycin was inhibitory at 7.5 μg/ml. Neomycin–polymyxin B agar was not inhibitory to the organism but was less than satisfactory since many facultative organisms also grew on it. The anaerobe grows well on blood agar plates with 100 μg/ml gentamicin (Black and Van Buskirk 1973) and produces characteristic beta hemolysis. This medium is highly selective and differential.

Carbohydrates were generally not fermented. Fructose, glucose, and lactose were weakly fermented (pH 5.5–6.0). Maltose and mannose were either weakly fermented or negative and are listed as variable. All other carbohydrates tested were not fermented (Table 1).

Fermentation products from PY–glucose were acetic acid (0.8 mEq/100 ml of culture) and traces of pyruvic and lactic acids. No acids were found in PY without glucose and no attempt was made to detect alcohols.

None of the strains produced catalase or lipase. Indole, ammonia, and hydrogen sulfide were not detected. Nitrate was not reduced to nitrite or volatile end products. Meat was not digested, gelatin not liquified, and starch not hydrolyzed.

All strains were deoxyribonuclease and lecithinase positive, and were beta hemolytic on sheep blood agar (Table 1).

Since *E. tarantellus* grew at 37°C, was hemo-

FIG. 2. Phase-contrast micrograph of *Eubacterium tarantellus* cells grown for 24 h in Brewer's thioglycollate medium. These long, unbranched filaments break up into shorter rods and chains during serial transfers. Bar = 10.0 $\mu$m.

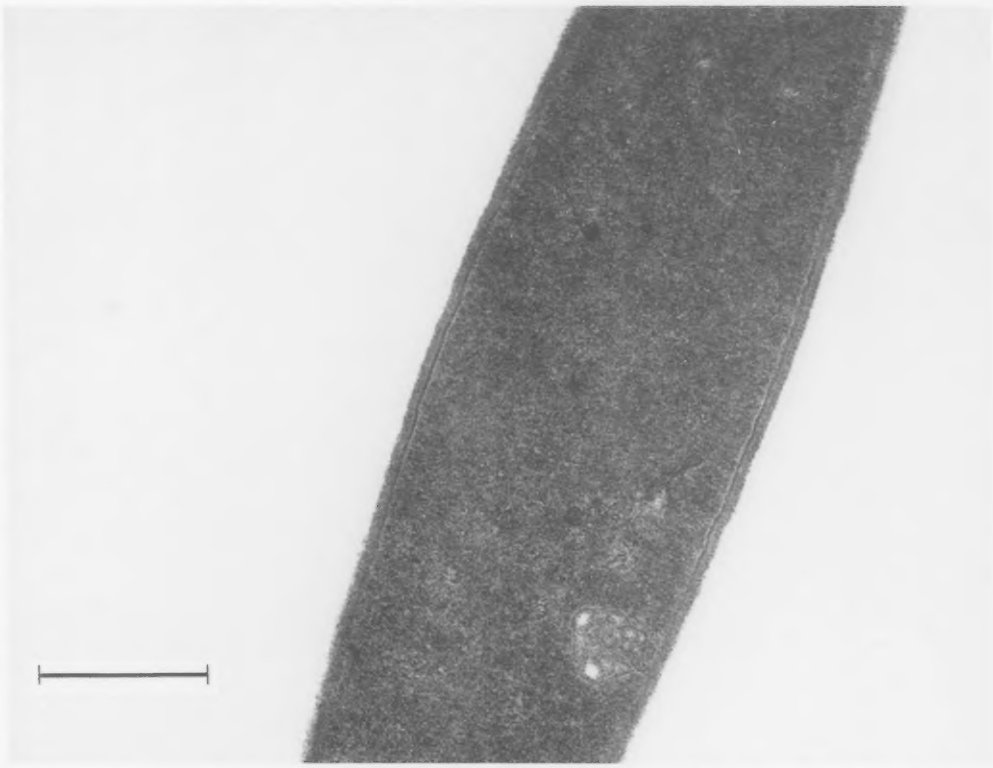

FIG. 3. Transmission electron micrograph showing the ultrastructure of a thinly sectioned *E. tarantellus* cell. Bar = 0.5 μm.

lytic, and produced deoxyribonuclease and lecithinase, it seemed likely that it might be pathogenic to mammals. Mice injected with CMM culture supernatant showed no signs of toxemia during 2 wk of examination. Guinea pigs inoculated with the organism showed no signs of edema, necrosis, or paralysis during the 21-day observational period nor did any of the animals die. Portions of each inoculum were subcultured in BTM to demonstrate that the inoculum had not been inactivated by suspension in saline or $CaCl_2$. Growth from both suspensions was luxuriant within 24 h at 25°C.

Channel catfish were used to determine if *E. tarantellus* was pathogenic for poikilothermic animals. In contrast to many of the estuarine fish sampled to date, this species has never been shown to harbor the anaerobe. Approximately 2 wk after inoculation with the organism, three of six fish died. The anaerobe was isolated from two of the three. No anaerobes were isolated from the six control fish or the three surviving experimental fish when necropsied at the end of the month.

## Discussion

Taxonomic designation: *Eubacterium tarantellus* sp.nov. (Ital. n. tar′an-tell′a) a fast, whirling dance [refers to the disease symptoms]; type strain (UM-87) ATCC 29255.

This organism was placed in the genus *Eubacterium* because it is a gram-positive, nonspore-forming, anaerobic rod that produces acetate or acetate with traces of lactate and pyruvate as the sole major fermentation products. It does not produce butyric acid.

Most *Eubacterium* species produce butyric acid; only 7 of the 32 currently accepted species do not. To these seven another four (unnamed) types were added by Moore and Holdeman (1974); of this combined group, the fish isolates most closely resemble *E. tenue*, *E. aerofaciens*,

TABLE 1. Physiological, biochemical, and pathogenic characteristics of *Eubacterium tarantellus* sp.nov. (ATCC 29255), from striped mullet (*Mugil cephalus*). A = final pH of the culture was < 5.5; W = final pH of the culture was between 6.0 and 5.5; a = acetic acid, <1 mEq/100 ml; py = pyruvate, 1 mEq/100 ml; l = lactic acid, <1 mEq/100 ml; ( ) = variable between strains; V = variable, only weak acid (W) or no acid (−) was produced depending upon the strain.

| Characteristic | Reaction (five strains) | Carbohydrate | Reaction (five strains) |
|---|---|---|---|
| Gram | + | amygdalin | − |
| Spores | − | arabinose | − |
| Motility | − | cellobiose | − |
| Growth temperature (C) | | | |
| range | 15–40 | esculin pH | − |
| optimum | 25 | esculin hydrolysis | − |
| 24-h survival | 4–42 | fructose | W |
| | | glucose | W |
| Growth salinity (‰) | | | |
| range | 0.5–2.0 | | |
| 24-h survival | 0.5–10.0 | lactose | W |
| | | maltose | V |
| Growth pH | | mannitol | − |
| range | 5.6–8.0 | mannose | V |
| 24-h survival | 3.4–8.8 | melezitose | − |
| $NH_4$ | − | | |
| $H_2S$ | − | | |
| Catalase | − | raffinose | − |
| DNAase | + | rhamnose | − |
| Gelatinase | − | | |
| Lecithinase | + | salicin | − |
| Lipase | − | | |
| Hemolysis | β | starch pH | − |
| Nitrate | − | starch hydrolysis | − |
| Indole | − | sucrose | − |
| Meat | − | | |
| Milk | A | trehalose | − |
| Peptone–yeast–glucose products (GLC) | a(py, l) | xylose | − |
| Mouse toxicity | − | | |
| Guinea pig pathogenicity | − | | |
| Catfish pathogenicity | + | | |

and *E. lentum*. All four species are similar in that they are not physiologically very active. Significant differences, however, can be found in their fermentation and biochemical reactions, culture characteristics, morphology, or pathogenicity.

Therefore, we suggest that these fish isolates be considered a new species and be given the name *E. tarantellus*.

It is not surprising that anaerobic bacterial pathogens of fish have not been described. Only recently have infections caused by anaerobes (other than *Clostridium* spp.) received much attention in human clinical laboratories. The magnitude and importance of anaerobic bacterial infections were not understood until after the widespread acceptance and use of standard techniques (Holdeman and Moore 1975; Sutter et al. 1975; Dowell and Hawkins 1974). Other developments such as the Gas-Pak anaerobic jar have greatly simplified and reduced the cost of culturing anaerobes. Fish pathologists should be able to make rapid advances in this area using the same methods currently being used for human specimens.

Since finding *E. tarantellus* infections in mullet, the microorganism has been isolated from the brains of 10 additional species of estuarine fish from Biscayne and Florida bays (unpublished data). Many marine fish that never enter the bays have also been examined and *E. tarantellus* has not been recovered in a single instance.

These observations, coupled with the fact that the organism does not grow at salt concentrations above 2%, suggest that it is limited to estuarine environments. The anaerobe appears to infect several but not all estuarine fishes. Additionally,

it has not been isolated from all fish of any one species. It can also be isolated from fish, including striped mullet, that do not display clinical (e.g. twirling) symptoms.

The anaerobe may grow slowly in the host and produce disease only after a protracted period. This is supported by our observations that the symptoms are seen primarily in mature fish. Alternatively, the disease may be chronic and subclinical and may only become acute when the fish is stressed.

In a recent paper, Henley and Lewis (1976) reported two strains of an anaerobic bacterium isolated from moribund fish taken off the Texas coast. They tentatively placed these strains in the genus *Catenabacterium* but this genus is no longer recognized (Holdeman and Moore 1974; Holdeman, personal communication, Virginia Polytechnic Institute, Blacksburg, Va.). Based upon the characteristics ascribed to these strains, they should be placed in the genus *Eubacterium*.

The Texas isolates differ in only a few characteristics from our strains. Furthermore, we have recently demonstrated by indirect immunofluorescence that both of the strains from Texas have antigens in common with *E. tarantellus* (unpublished data). Although further clarification is needed, it is likely that the Florida and Texas anaerobes are closely related, if not biovariants of the same species.

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# Resting Eggs in *Pontella meadi* (Copepoda: Calanoida)[1]

GEORGE D. GRICE AND VICTORIA R. GIBSON

*Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543, USA*

GRICE, G. D., AND V. R. GIBSON. 1977. Resting eggs in *Pontella meadi* (Copepoda: Calanoida). J. Fish. Res. Board Can. 34: 410–412.

*Pontella meadi* Wheeler produces resting eggs in fall which hatch the following summer. Experiments show that these eggs require 4–8 wk of incubation at 2–3 or 5–6°C for substantial hatching to occur. Eggs occur in sediment in winter. Resting eggs serve to repopulate temperate inshore areas with this species after its winter disappearance from the plankton.

*Key words*: Copepoda, Calanoida, *Pontella*, resting eggs, incubation time, distribution

GRICE, G. D., AND V. R. GIBSON. 1977. Resting eggs in *Pontella meadi* (Copepoda: Calanoida). J. Fish. Res. Board Can. 34: 410–412.

*Pontella meadi* Wheeler produit des œufs dormants en automne qui éclosent l'été suivant. Des expériences démontrent que ces œufs requièrent de 4 à 8 semaines d'incubation à 2–3°C ou à 5–6°C pour qu'il y ait éclosion substantielle. Les œufs se trouvent dans le sédiment en hiver. Les œufs dormants servent à repeupler les régions côtières tempérées après que l'espèce ait disparu du plancton en hiver.

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IN the description of the developmental stages of *Pontella meadi* Wheeler, Gibson and Grice (1976) noted that the first occurrence of this species near Woods Hole, the northern part of its range, in summer (July), could be attributed either to recruitment from offshore waters or to the hatching of local resting eggs. Here we elucidate the mechanism whereby it repopulates and maintains itself in the Woods Hole area, which, since *P. meadi* occurs between Cape Cod and Cape Hatteras during the same period (summer and fall), will probably account for its distribution in temperate inshore waters of the western North Atlantic.

*Materials and methods* — Eggs of *P. meadi* were obtained from females collected in Vineyard Sound and Buzzards Bay (41°32′N, 70°45′W), July through November 1975. Individual adult females were isolated from neuston collections, placed in 125-ml dishes, fed day old *Artemia*, and allowed to remain overnight at 20°C. Since adults can jump a considerable distance out of the water, they may adhere to the undersurface of dish lids and die. To avoid this, a watch glass was positioned over each dish so that the convex side was in contact with the water and air spaces were eliminated. The first clutch laid by each female, usually consisting of 50–130 eggs, was used for experiments. Eggs were transferred by pipette to 75-ml jars which were either maintained in the laboratory at a constant temperature (2, 6, and 15°C), or suspended beneath the Institution pier at ambient water temperature in Woods Hole Harbor. For determining egg viability, jars were gradually warmed to 20°C for most experiments and daily counts were made of hatched nauplii. On March 19 sediment samples from a depth of approximately 5 m were collected in Vineyard Sound with an Ekman grab.

*Results* — Eggs laid in July and August usually hatched within 48 h at 20°C and within 96 h at 15°C. Viability, as reflected by hatching of nauplii, was reduced when eggs were incubated at 6 and 2°C. Eggs incubated at 6°C for a prolonged period sometimes hatched at that temperature; eggs kept at 2°C had to be warmed for hatching to occur, and viability was substantially less than when they were first laid (Table 1). After incubation for 100 days or more at 2°C, eggs were nonviable in 11 of 18 test batches and $< 50\%$

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TABLE 1. Hatching of summer eggs after incubation at 2–3°C[a].

| Clutch | Initial viability[b] | Days incubated | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 40 | 50 | 75 | 83 | 100 | 125 | 150 | 175 | 200 | 225 | 250 |
| 1 | 10(10) | | | | 10(20) | 3(20) | | | 0(18) | | | |
| 2 | 10(10) | | | | 4(10) | 5(10) | | | 0(10) | 0(10) | | |
| 3 | 8(10) | | 30(35) | | | | | | | | | |
| 4 | 10(10) | | | 6(10) | | 5(10) | | 2(10) | | 0(10) | | 0(9) |
| 5 | 10(10) | 14(24) | | | | | | | | | | |
| 6 | 10(10) | | | 6(10) | | 5(10) | 3(10) | 5(10) | | | 0(10) | |
| 7 | 9(10) | | | | | | | 0(16) | | | | |
| 8 | 6(10) | | | | | | | 0(15) | | | | |
| 9 | 7(10) | | | | | | | 0(15) | | 0(5) | 0(15) | |
| 10 | 10(10) | 5(20) | | | | | | | | | | |

[a]Numbers shown are number that hatched of the number in parentheses after warming to 20°C.
[b]Based on number hatched when maintained at 20°C.

viable in 3 batches. No summer eggs hatched when incubated longer than 150 days.

Females continued to lay through November, but eggs laid after mid-September, unlike summer eggs, did not readily hatch at ambient temperatures (from 20°C in September to 13°C in November), even when the temperatures were those at which summer eggs hatch (20°C). These fall eggs are considered resting eggs which require a period of reduced temperature followed by warming before hatching will occur. In laboratory incubators it required about 30 days at 2–3°C for 50% of the clutch to hatch and about 60 days at 5–6°C (Table 2) for 50% of the clutch to hatch after warming to 20°C. Eggs incubated in Woods Hole Harbor (temperature 3–10°C) did not hatch after 30 days but all hatched after 60 days following warming to 20°C. The 30-day group of eggs were placed in an incubator (2–3°C) and all hatched after 30 more days of incubation. After 90 days of laboratory incubation at 6°C, eggs hatched in 50–76 days when the temperature was raised to 10–12°C. When incubation took place in Woods Hole Harbor from October to April (about 210 days), substantial hatching occurred 2 wk after the eggs were warmed to 10–12°C. No difference was found in hatching rate between eggs maintained in light and darkness.

Of two clutches of eggs incubated in Woods Hole Harbor, eggs of the first cluch were 100% viable for 6 mo and those of the second were 70% or more viable for 5 mo (Table 3). In the second clutch none of the eggs hatched after 180 days, but all eggs in a sample of eight from the same clutch maintained at 2–3°C in a laboratory incubator hatched after 180 days.

In general, resting eggs maintained in incubators and in Woods Hole Harbor retained high viability for as long as 210 days (Table 4).

TABLE 2. Hatching of resting eggs incubated at 2–3, 5–6 C, and in Woods Hole Harbor at ambient temperature.

| Days | No. eggs | No. hatched[a] | Days | No. eggs | No. hatched[a] |
|---|---|---|---|---|---|
| | 2–3°C | | | 5–6°C | |
| | *Clutch 1* (laid Oct. 15) | | | *Clutch 1* (laid Oct. 15) | |
| 5 | 10 | 0 | 5 | 10 | 0 |
| 10 | 10 | 2 | 15 | 10 | 1 |
| 30 | 10 | 6 | 30 | 10 | 0 |
| 60 | 10 | 10 | 60 | 10 | 9 |
| | *Clutch 2* (laid Oct. 15) | | | *Clutch 2* (laid Oct. 15) | |
| 5 | 10 | 1 | 5 | 10 | 0 |
| 15 | 10 | 0 | 15 | 10 | 0 |
| 30 | 10 | 5 | 30 | 10 | 2 |
| 60 | 10 | 9 | 60 | 10 | 9 |
| | *Clutch 3* (laid Oct. 24) | | | | |
| 4 | 5 | 0 | | *In situ*[b] | |
| 8 | 5 | 0 | | *Clutch 1* | |
| 12 | 5 | 2 | | (laid Oct. 24) | |
| 16 | 5 | 2 | 30 | 10 | 0 |
| 20 | 5 | 0 | 60 | 10 | 10 |
| 24 | 5 | 1 | 90 | 10 | 10 |
| 28 | 5 | 4 | | | |

[a]After warming to 20°C.
[b]Woods Hole Harbor at depth of 12 m, temperature 16.5°C at day 0, 10°C at day 30, 3°C at day 60, 0°C at day 90.

Sediment that was collected in March and incubated at 20°C yielded *P. meadi* nauplii.

*Conclusions* — *Pontella meadi* in the Woods Hole area produce two types of eggs: summer

TABLE 3. Hatching of resting eggs incubated in Woods Hole Harbor.

| Incubation (*days*) | Last day of incubation | Water temp. (°C) on last day of incubation | No. eggs | No. hatched[a] |
|---|---|---|---|---|
| Clutch 1 (laid Oct. 16) | | | | |
| 60 | Dec. 20 | 3 | 6 | 6 |
| 90 | Jan. 19 | 0 | 8 | 8 |
| 120 | Feb. 18 | 1 | 8 | 8 |
| 150 | Mar. 20 | 4 | 10 | 10 |
| 180 | Apr. 19 | 10 | 12 | 12 |
| 210 | May 19 | 13 | 10 | 9 |
| Clutch 2 (laid Oct. 27) | | | | |
| 30 | Nov. 26 | 10 | 10 | 10 |
| 60 | Dec. 26 | 3 | 10 | 10 |
| 90 | Jan. 25 | 0 | 10 | 10 |
| 120 | Feb. 24 | 3 | 10 | 10 |
| 150 | Mar. 25 | 4 | 10 | 7 |
| 180 | Apr. 24 | 10 | 10 | 0 |

[a]After warming to 20°C.

TABLE 4. Hatching of resting eggs after prolonged incubation.

| 2–3°C[a] | 5–6°C[a] | Woods Hole Harbor[a] |
|---|---|---|
| 175 days | 175 days | 175 days |
| 25 (25) | 23 (25) | 25 (25) |
| 10 (10) | 10 (10) | 0 (10)[b] |
| 201 days | 202 days | 203 days |
| 1 (14) | 11 (14) | 10 (14) |
| 5 (10) | 8 (10) | 7 (10) |
| 210 days | 210 days | 210 days |
| 35 (40) | 35 (40) | 9 (10) |

[a]Numbers shown are number that hatched of the number in parentheses after warming to 20°C.
[b]180 days.

eggs, laid in July and August, hatch at ambient temperature and exhibit a sharp decrease in viability when incubated for several months at 2–3°C; winter, or resting eggs, laid from September to November, require a period of reduced temperature before hatching and retain high viability for up to 6 mo. Since resting eggs have been shown to occur in bottom sediment in winter, the first occurrence of adult *P. meadi* in inshore temperate neritic waters in summer apparently results from hatching of eggs laid the preceding fall, as has been demonstrated for *Labidocera aestiva*, another pontellid copepod (Grice and Gibson 1975).

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# A Red Tide Caused by the Marine Ciliate *Mesodinium rubrum* in Passamaquoddy Bay, Including Pigment and Ultrastructure Studies of the Endosymbiont

ALAN W. WHITE

*Department of Fisheries and the Environment, Fisheries and Marine Service, Biological Station, St. Andrews, N.B. E0G 2X0*

ROBERT G. SHEATH AND JOHAN A. HELLEBUST

*Department of Botany, University of Toronto, Toronto, Ont. M5S 1A1*

WHITE, A. W., R. G. SHEATH, AND J. A. HELLEBUST. 1977. A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont. J. Fish. Res. Board Can. 34: 413–416.

A red tide caused by *Mesodinium rubrum* was observed in Passamaquoddy Bay, New Brunswick, during August 1975. The chlorophyll and phycoerythrin composition and ultrastructure of the endosymbiotic alga are similar to those described for *M. rubrum* from Ecuador and British Columbia, including the fact that the endosymbionts do not appear to contain a nucleus. Special ultrastructural features are a complete endoplasmic reticulum surrounding symbiont chloroplast, pyrenoid, and starch bodies, ciliate trichocysts with electron-dense and electron-translucent regions, and curious symbiont mitochondria–ciliate macronuclei associations.

WHITE, A. W., R. G. SHEATH, AND J. A. HELLEBUST. 1977. A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont. J. Fish. Res. Board Can. 34: 413–416.

On a observé en août 1975 dans la baie Passamaquoddy, Nouveau-Brunswick, une marée rouge causée par *Mesodinium rubrum*. La composition en chlorophylle et en phycoérythrine, ainsi que l'ultrastructure de l'algue endosymbiotique sont semblables à celles décrites chez *M. rubrum* de l'Equateur et de la Colombie-Britannique, y compris le fait que les endosymbiontes ne semblent pas contenir de noyau. Comme caractéristiques ultrastructurales spéciales, on note un reticulum endoplasmique complet entourant le chloroplaste, les pyrénoïdes et les corps d'amidon du symbionte; des trichocystes du cilié, avec régions opaques et translucides aux électrons; et de curieuses associations mitochondries du symbionte–macronuclei du cilié.

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*Mesodinium rubrum* (Lohmann) is a planktonic marine ciliate responsible for red tide conditions in many coastal areas of the world (Taylor et al. 1971). Although the brick-red blooms of this ciliate do not appear to be toxic or noxious to other marine life, the organism itself is fascinating because of the unusual symbiosis between a ciliate and an alga to the point that functionally the organism acts as an autotroph (Ryther 1967; Barber et al. 1969). The taxon is probably conspecific with *Cyclotrichium meunieri* (Powers) (Taylor et al. 1971; Powers 1932), and the taxa are considered to be the same here.

Studies of pigment composition on specimens from Ecuador (Barber et al. 1969) and from British Columbia (Parsons and Blackbourn 1968) led to the conclusion that the endosymbiotic alga causing the red color of *M. rubrum* is probably a cryptomonad. Ultrastructural studies of *M. rubrum* from British Columbia (Taylor et al. 1969 and 1971) showed that the chloroplast thylakoid structure of the endosymbiont is unlike that of the few other cryptomonads examined, and that the symbionts do not appear to contain nuclei, which raises many questions concerning the relationship between the ciliate and its symbionts. A review of the literature on *M. rubrum* blooms and a discussion of the functional and evolutionary significance of its "incomplete symbionts" is presented by Taylor et al. (1971).

During the last week of August 1975, red water due to *M. rubrum* was observed near the surface in Passamaquoddy Bay. The bloom provided an opportunity to examine *M. rubrum* and its symbionts in a population geographically remote from those previously studied in Peru–Ecuador and British Columbia. This paper gives

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an account of the bloom and results of pigment and ultrastructure studies.

*Materials and methods* — Bucket samples of the red water were collected and returned to the laboratory as soon as possible and the causative organism was identified as *Mesodinium rubrum*. Bary and Stuckey (1950) and McAlice (1968) observed that, when exposed to bright light, the ciliates quickly formed swarms near the bottom and sides of the bucket. This response was used to concentrate the organisms for fixation.

The ciliate was very delicate and rapidly burst on exposure to formalin or on a microscope slide under a cover slip. Fixatives tried were formalin, formalin–acetic acid, ethanol, Bouin's solution, glutaraldehyde, buffered glutaraldehyde–acetic acid, buffered glutaraldehyde–sucrose, sucrose, and osmium tetroxide. The best results in terms of the shape of the ciliate and its ciliary band, and retention of the water-soluble red pigment were obtained with a 2-h fixation with 1% $OsO_4$ in 0.05 M sodium cacodylate at pH 7 and at 4 C. The cells were then dehydrated through a graded ethanol–acetone series, using 10% increments, embedded in a 4-step infiltration of Spurr's low-viscosity resin, and hardened for 12 h at 70°C (Spurr 1969). Sections were cut on a Reichert Ultramicrotome OmU2, stained with Reynolds' lead citrate (Reynolds 1963) for 5 min, and then viewed with a Zeiss EM9 electron microscope.

Pigment analysis was performed according to the methods described by Barber et al. (1969). Absorption spectra were made with a Beckman DB-GT recording spectrophotometer.

*Results and discussion* — During the week the patch of red water drifted from the area of Western Passage (44°57′N, 67°01′W) to the area of Tongue Shoal (45°03′N, 67°01′W). The general dimensions of the patch were about 20–50 m wide and 1–2 km long, although it was broken up into several lobes; water color was a deep, brick red. Surface water temperature was 13.5°C, and surface salinity was 31.5–32.0‰.

The ciliates were small, 30–50 μm across, and swam in a darting, zig-zag fashion. On dying they changed rapidly from their normal sandy red to a yellowish green.

The pigment composition of *M. rubrum* from Passamaquoddy Bay, with respect to chlorophylls and water-soluble red pigment, was the same as that described for *M. rubrum* from Peru (Ryther 1967), Ecuador (Barber et al. 1969), and British Columbia (Parsons and Blackbourn 1968). Absorption maxima of the hexane layer only at 662, 620, and 431 nm indicated the presence of chlorophyll *a* and the absence of chlorophyll *b*. A shoulder at 478 nm was probably due either to α-carotene or an unnamed orange pigment (Parsons and Blackbourn 1968). The presence of chlorophyll *c* was confirmed by absorption maxima of the hexane-washed acetone layer at 632, 582, and 444 nm (Bogorad 1962). Also, upon acidification the peaks at 632 and 582 nm were eliminated, and the peak at 444 nm was shifted to 430 nm, characteristic of chlorophyll *c*.

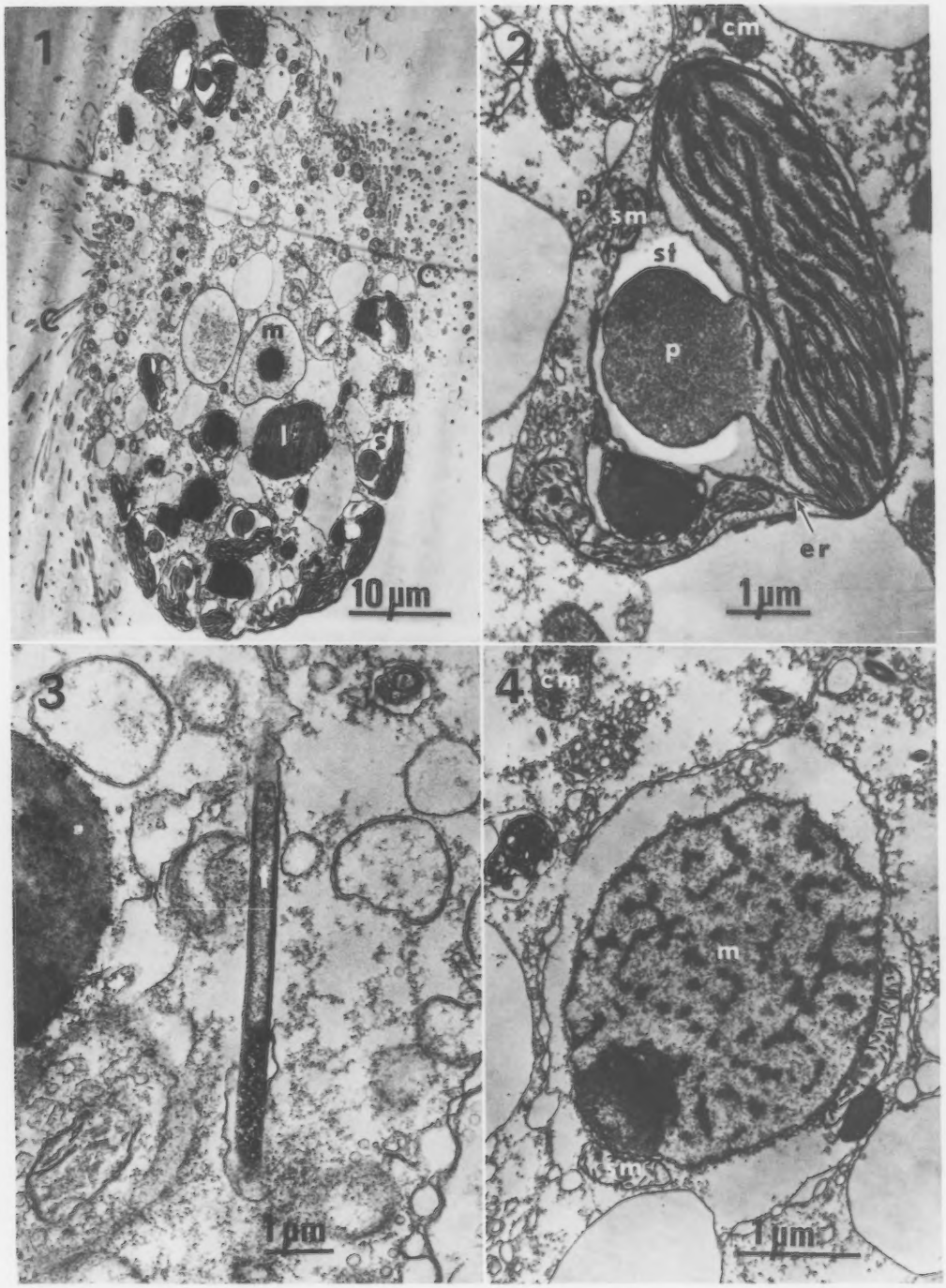
The absorption spectrum of the water-soluble red pigment was identical to that shown for *M. rubrum* from Ecuador (Barber et al. 1969), except that the absorption maximum was recorded at 544 nm for the Passamaquoddy Bay form as opposed to 542 nm for the Ecuador form. This small difference may be real or may be due to calibration errors in the spectrophotometers. The single absorption maximum at 544 nm is consistent with the contention of Barber et al. (1969) that the water-soluble red pigment is a phycoerythrin (Goodwin 1974; ÓhEocha 1962). Phycoerythrins, which are biliproteins, are found only among the red algae, blue-green algae, and cryptomonads. Since chlorophylls *a* and *c* are present along with the phycoerythrin, then the endosymbiont of *M. rubrum* is probably of cryptomonad origin (Barber et al. 1969).

The ultrastructural details of *M. rubrum* and its symbionts from Passamaquoddy Bay were also similar to those recently described for *M. rubrum* from British Columbia and Peru (Taylor et al. 1969 and 1971). A typical longitudinal section of the ciliate from Passamaquoddy Bay is given in Fig. 1, showing the equatorial ciliary band, posterior ring of cirri, macronuclei, and anteriorly and posteriorly located endosymbionts. Figure 2 shows a symbiont with its plasmalemma, chloro-

FIGURES 1–4

Symbols used: m = macronucleus, s = symbiont, l = lipid droplet, c = cilia, t = trichocyst, pl = plasmalemma p = pyrenoid, st = starch, sm = symbiont mitochondrion, cm = ciliate mitochondrion, er = endoplasmic reticulum.

FIG. 1. A near longitudinal section of *Mesodinium rubrum* showing macronuclei ciliary arrangement, and localization of symbionts. Fig. 2. Longitudinal section through a symbiont with chloroplast containing lamellae in stacks of three, and with a stalked pyrenoid surrounded by starch. The chloroplast, pyrenoid, and starch are enclosed by endoplasmic reticulum. Peripheral to this are lipid droplets and mitochondria. The symbiont is enveloped by a plasmalemma. FIG. 3. Longitudinal section of a ciliate trichocyst showing electron-dense and electron-translucent regions. FIG. 4. Section of a ciliate macronucleus with symbiont mitochondria and lipid droplet in close proximity. This association is surrounded by endoplasmic reticulum.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plast with lamellae in loose stacks of three, stalked pyrenoid, starch bodies, mitochondria, and complete endoplasmic reticulum surrounding the chloroplast, pyrenoid, and starch. Interestingly, we have corroborated the observations of Taylor et al. (1969 and 1971) on the apparent lack of nuclei associated with endosymbionts of *M. rubrum*.

Trichocysts were comomnly seen in the ciliates. Figure 3 shows a longitudinal view of a trichocyst with electron-dense and electron-translucent ends. The trichocyst is partially covered by a single-membrane sheath.

A most unexpected observation was the frequent occurrence of symbiont mitochondria in close association with ciliate macronuclei, both of which were surrounded by a double-membrane system, possibly of endoplasmic reticulum origin (Fig. 4). The significance of this association is not clear. It is possible that the endosymbiont undergoes a sequence of degradation after initial ingestion by the ciliate, and that the nuclei and flagella of the symbiont are rapidly lost while the chloroplast and other organelles persist for relatively long periods. The fact that there are free symbiont mitochondria associated with ciliate macronuclei indicates a further step in symbiont degradation.

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# Nitrogen Fixation (Acetylene Reduction) Associated with Sea Urchins (*Strongylocentrotus droebachiensis*) Feeding on Seaweeds and Eelgrass

M. L. GUERINOT, W. FONG, AND D. G. PATRIQUIN

*Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1*

GUERINOT, M. L., W. FONG, AND D. G. PATRIQUIN. 1977. Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding on seaweeds and eelgrass. J. Fish. Res. Board Can. 34: 416–420.

Sea urchins feeding in different macrophyte zones in St. Margaret's Bay, Nova Scotia, in November and December 1975 were examined for nitrogenase activity using the acetylene reduction technique. For sea urchins feeding on *Laminaria digitata* and *L. longicruris*, the average rate of acetylene reduction was equivalent to $N_2$ fixation of 55 $\mu$g $N_2$ per sea urchin per day assuming a 3:1 molar ratio of $C_2H_2$ reduction to $N_2$ fixation. Lower nitrogenase activities were observed for sea urchins feeding on *Agarum cribrosum* and *Zostera marina*, and no

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nitrogenase activity was observed for sea urchins taken from bare rock substrate on which kelp had been absent for 4 yr. Samples of separated digestive tracts but not the degutted bodies of sea urchins exhibited nitrogenase activity, suggesting that the digestive tract is the site of nitrogen fixation in whole sea urchins.

GUERINOT, M. L., W. FONG, AND D. G. PATRIQUIN. 1977. Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding on seaweeds and eelgrass. J. Fish. Res. Board Can. 34: 416–420.

Nous avons examiné, utilisant la technique de réduction de l'acétylène, des oursins se nourrissant dans différentes zones de macrophytes dans la baie St. Margaret, Nouvelle-Ecosse, en novembre et décembre 1975, en vue d'y détecter une activité de nitrogénase. Chez des oursins se nourrissant de *Laminaria digitata* et de *L. longicruris*, le taux moyen de réduction de l'acétylène équivaut à une fixation de $N_2$ de 55 $\mu$g $N_2$ par oursin par jour, en supposant un rapport molaire réduction de $C_2H_2$: fixation de $N_2$ de 3:1. On observe des activités de nitrogénase moindres chez des oursins se nourrissant d'*Agarum cribrosum* et de *Zostera marina*, et aucune activité de nitrogénase chez des oursins prélevés sur un substrat de rocher dénudé d'où les varechs étaient absents depuis 4 an. Des échantillons de tractus digestif isolé, mais non de corps éviscéré d'oursins montrent une activité de nitrogénase, ce qui donne à croire que le tractus digestif est le site de fixation d'l'azote dans des oursins entiers.

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IN Eastern Canada, sea urchins (*Strongylocentrotus droebachiensis*) are found feeding primarily on seaweeds (Himmelman and Steele 1971; Miller and Mann 1973) with a few feeding on eelgrass. Sea urchin grazing has, in fact, been reported to limit the local distribution of both seaweeds and seagrasses in many parts of the world. The destructive grazing of kelp by sea urchins in St. Margaret's Bay, Nova Scotia, is believed to be related to declining populations of lobsters (*Homarus americanus*), a major predator of sea urchins (Breen and Mann 1976). Marine macrophytes are commonly nitrogen poor relative to the nutritional requirement of grazing invertebrates (Mann 1972). Using the acetylene reduction assay for nitrogenase activity, we investigated the possibility that $N_2$ fixation occurs in sea urchins, thus possibly supplementing their dietary intake of nitrogen. Although $N_2$ fixation ($C_2H_2$ reduction) has been demonstrated to occur in marine shipworms feeding on woody tissue (Carpenter and Culliney 1975), it has not been reported for invertebrates feeding on marine plants, which have much lower C:N ratios.

*Methods* — Sea urchins (*S. droebachiensis*) were collected in November and December 1975 from the following zones in St. Margaret's Bay (Mann 1971); i) *Agarum cribrosum*, ii) *Laminaria digitata* with *L. longicruris*, iii) *Zostera marina*, iv) no macrophytes. Acetylene reduction assays for nitrogenase activity (Hardy et al. 1968) were initiated in the field. Animals were incubated in 110- or 450-ml jars filled to 1/10 of their volumes with sea water and closed with caps in which serum stoppers had been inserted. After injection of freshly generated acetylene ($pC_2H_2$ about 0.1 atm) the samples were held in ice chests maintained within ± 2°C of sea temperatures and within 2½ h were transferred to laboratory incubators maintained within ± 0.5°C of sea temperatures.

On each sampling occasion, acetylene reduction assays for nitrogenase activity were carried out as above on sea water, and on fresh plant materials of the type on which the sea urchins were feeding (sea urchins were always dissected at the end of every experiment to verify their major food source).

During the course of the studies, sea urchins from the kelp zone were dissected and the digestive tract (with contents) as well as the degutted bodies (digestive tract and contents removed) were assayed separately.

Gas phase samples, 0.5 ml, were analyzed for $C_2H_4$ and $C_2H_2$ by use of a Hewlett Packard model 5710A gas chromatograph equipped with a 50 × 0.23-cm column containing Poropak T and with flame ionization detectors. The instrument was operated at an oven temperature of 50°C, detector temperature of 100°C, and $N_2$ carrier gas flow rate of 25 ml/min. Retention times for $C_2H_4$ and $C_2H_2$ were 19 and 35 s, respectively. Ethylene content of injected samples was determined from standard curves of peak height vs. known $C_2H_2$ concentrations. Replicate samples were within 2% of the mean. Nitrogen content of macrophytes was determined by the Kjeldahl technique. Duplicate samples were within 5% of the mean.

*Results* — High rates of $C_2H_4$ production were observed for many of the sea urchin samples in the presence of $C_2H_2$ but not in its absence and are attributed to nitrogenase activity. Rates of $C_2H_4$ production remained relatively constant over incubation periods up to 30 h (Fig. 1) and for comparisons we have estimated nitrogenase activity from the increase in $C_2H_4$ production be-

TABLE 1. Summary of nitrogenase activities of live sea urchins (*Strongylocentrotus droebachiensis*) collected from St. Margaret's Bay, N.S.

| Date (1975) | Water temp (°C) | Feeding area | Number sampled | Number positive | Nitrogenase activity[a] mean | range ($\mu$mol $C_2H_4$·animal$^{-1}$·day$^{-1}$) | SD |
|---|---|---|---|---|---|---|---|
| Nov. 17 | 9 | *Laminaria digitata* and *L. longicruris* | 9 | 9 | 2.16 | 0.460 to 4.287 | 1.67 |
| Nov. 17 | 9 | Rock area, edge of kelp bed | 9 | 9 | 0.68 | 0.049 to 1.059 | 1.37 |
| Dec. 3 | 6 | *L. digitata* and *L. longicruris* | 10 | 10 | 0.22 | 0.013 to 0.589 | 0.22 |
| Dec. 3 | 6 | Rock area, no kelp for 4 yr | 10 | 0 | 0. | 0 to 0 | 0. |
| Dec. 14 | 4 | *L. digitata* and *L. longicruris* | 13 | 12 | 5.92 | 0 to 14.925 | 4.47 |
| Dec. 14 | 4 | *Zostera marina* | 18 | 7 | 1.12 | 0 to 7.686 | 2.23 |
| Dec. 14 | 4 | *Agarum cribrosum* | 17 | 4 | 0.85 | 0 to 11.001 | 2.74 |

[a]Ethylene production greater than 2 times that present in 0.1 atm freshly generated $C_2H_2$.

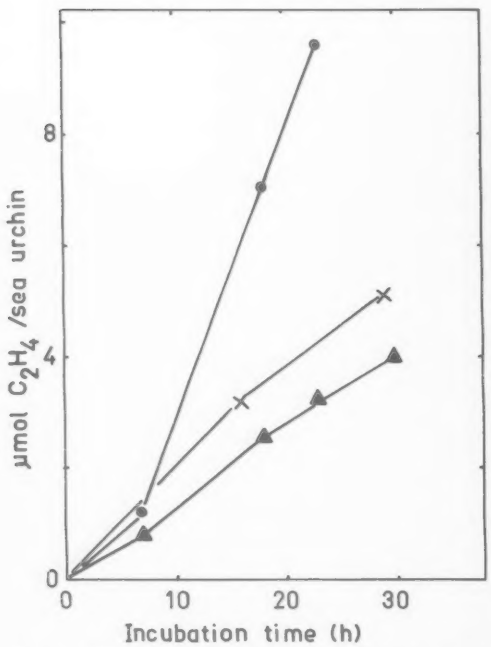


FIG. 1. Representative time courses of acetylene reduction by live sea urchins (*Strongylocentrotus droebachiensis*) collected from different macrophyte zones: (●) *Laminaria* spp. zone, December 3, (×) *Agarum cribrosum* zone, December 14, (▲) *Zostera marina* zone, December 14, 1975.

tween approximately 7 and 18 h after initiation of the incubations.

For whole urchins, nitrogenase activities varied widely within samples and between samples collected from the different feeding areas (Table 1). Fresh plant material collected from each of these areas exhibited no nitrogenase activity. Sea urchins from the *Laminaria* spp. zone were examined on three occasions; on the second occasion (December 3) the nitrogenase activities were anomalously low in comparison to previous and subsequent samplings. Water (and incubation) temperature in the three experiments decreased from 9 to 4°C without any marked effect on nitrogenase activity.

There were significant differences, however, both in the number of sea urchins showing positive results and in the rates of $C_2H_2$ reduction among sea urchins collected from different feeding areas. Of the urchins tested on December 14, 92% of those feeding on *Laminaria* spp. but only 44% of those feeding on *Z. marina* and 24% of those feeding on *A. cribrosum* exhibited detectable nitrogenase activity. Animals collected from rocky areas where kelp beds have been absent for 4 yr showed no nitrogenase activity.

Acetylene reduction rates for sea urchins from different feeding areas are not directly comparable on a per sea urchin basis as their mean size differed in the various areas (Table 2). Therefore, a comparison on a per gram dry digestive tract (with contents) basis may be more appropriate. Paired *t*-tests showed that for sea urchins collected on December 14 (Table 2) there was a significant difference ($p < 0.01$) in rates of $C_2H_2$ reduction between sea urchins from the *Laminaria* spp. zone and those from the *Agarum* zone, but the differences in rates for sea urchins between the *Laminaria* spp. and the *Zostera* zones, and between the *Zostera* and the *Agarum* zones were not significant at the 0.05 level. It may be noteworthy that the highest average rates of $C_2H_2$ reduction were associated with sea urchins feeding on the macrophytes, *Laminaria* spp., having the lowest nitrogen contents (Table 2).

When the digestive tracts (and contents) and

TABLE 2. Comparison of nitrogenase activities for sea urchins taken from different macrophyte zones on December 14, 1975.

| Feeding area | % N of dry wt macrophyte[a] | Sea urchin diam. (*cm*) | | Nitrogenase activity | | |
|---|---|---|---|---|---|---|
| | | mean | range | mean ($\mu$mol $C_2H_4 \cdot g\ gut^{-1} \cdot day^{-1}$) | range | SD |
| *L. digitata* & *L. longicruris* | 0.87, 0.87, 0.94<br>1.69, 1.70, 1.69 | 4.84 | 4.01 to 5.51 | 6.86 | 0 to 15.0 | 5.23 |
| *Z. marina* | 2.87, 2.69 | 3.04 | 1.69 to 4.69 | 4.15 | 0 to 40.8 | 10.40 |
| *A. cribrosum* | 3.00, 2.80 | 2.88 | 2.17 to 4.37 | 1.21 | 0 to 12.8 | 3.50 |

[a]Data represent means of duplicate samples; samples were taken from different plants and different parts of plants.

the degutted bodies of sea urchins were assayed separately for nitrogenase activity, degutted bodies exhibited no nitrogenase activity while the separated digestive tracts exhibited nitrogenase activities of 0–0.281 $\mu$mol $C_2H_4 \cdot animal^{-1} \cdot day^{-1}$. These values are low in comparison to whole urchins (Table 2). As anaerobic or facultatively anaerobic bacteria might be responsible for the nitrogenase activity and the nitrogenase is inactivated by exposure to oxygen (Burns and Hardy 1975), we also incubated the digestive tract samples under 5 and 10% $O_2$ in $N_2$ and under $N_2$ and on one occasion the sea urchins were dissected and prepared under $N_2$ in a glovebag. These procedures failed to increase the rates of $C_2H_4$ production.

*Discussion* — This study demonstrates the occurrence of nitrogenase activity in whole sea urchins feeding on seaweeds and seagrasses. Although the nitrogenase activity of the separated digestive tracts were low in comparison to those for whole urchins, it was demonstrable, while degutted urchins and sea water exhibited no nitrogenase activity. This suggests that the digestive tract is the site of nitrogenase activity in the whole urchin. Breznak et al. (1973) have also reported that separated guts from termites exhibited low nitrogenase activity in comparison to the intact insect. As the handling of the sea urchins under $N_2$ did not increase the separated digestive tract nitrogenase activity, factors other than $O_2$ poisoning may be responsible for the low activity of the separated digestive tracts. Possibly $NH_4^+$ accumulates in separated digestive tracts, while it does not in the intact animal, and thus represses (Burns and Hardy 1975) nitrogenase activity. Since no measurable nitrogenase activity occurred in fresh plant materials (at ambient $O_2$ levels), the sea urchin digestive tract presumably provides an appropriate $N_2$-fixing microflora, and/or suitable environmental conditions for both the enrichment and activity of $N_2$-fixing bacteria.

Whether the $N_2$ fixed in the digestive tract is actually utilized by the sea urchin remains to be determined. The only nitrogen budget reported for a sea urchin is that given by Fuji and Kawamura (1970) for *S. intermedius*. They estimated the average daily nitrogen requirement to be between 365 and 712 $\mu$g N/animal. Assuming a 3:1 molar ratio of acetylene reduced to $N_2$ fixed (Hardy et al. 1968), the average rate of $N_2$ fixation for *S. droebachiensis* feeding on *Laminaria* spp. at 4°C on December 14 was about 55 $\mu$g $N \cdot animal^{-1} \cdot day^{-1}$, and the maximum $N_2$ fixation was 139 $\mu$g $N \cdot animal^{-1} \cdot day^{-1}$. If these data are at all comparable, then they suggest sea urchins in kelp beds could receive about 8–15% of their daily nitrogen requirements from $N_2$ fixation. Nitrogen budgets for sea urchins or other marine herbivores should take into consideration the posibility of $N_2$ fixation in the digestive tract.

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# Effects of Chemical Additives on Extending the Shelf Life of Frozen Minced Sucker (Catostomidae) Flesh

D. G. IREDALE AND R. K. YORK

*Department of Fisheries and the Environment, Fisheries and Marine Service, Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6*

IREDALE, D. G., AND R. K. YORK. 1977. Effects of chemical additives on extending the shelf life of frozen minced sucker (Catostomidae) flesh. J. Fish. Res. Board Can. 34: 420–425.

A 44-day accelerated storage test at −4°C was used to evaluate the relative effects of sodium erythorbate, disodium EDTA, and sodium tripolyphosphate on the development of oxidative rancidity in samples of minced sucker. Sensory testing and 2-thiobarbituric acid values were used to evaluate the changes in the samples. Sodium erythorbate was found to inhibit the development of oxidative rancidity as determined chemically and to contribute to maintaining a higher flavor score in the sensory testing.

IREDALE, D. G., AND R. K. YORK. 1977. Effects of chemical additives on extending the shelf life of frozen minced sucker (Catostomidae) flesh. J. Fish. Res. Board Can. 34: 420–425.

Dans une expérience d'entreposage accéléré de 44 jours à −4°C, nous avons évalué les effets relatifs de l'érythorbate de sodium, d'une solution bisodique d'EDTA et du tripolyphosphate de sodium sur le développement de la rancidité par oxydation dans des échantillons de catostome hachés. On s'est servi de tests sensoriels et des valeurs de l'acide 2-thiobarbiturique pour évaluer les changements dans les échantillons. L'érythorbate de sodium inhibe le développement de la rancidité par oxydation, telle que déterminée chimiquement, et contribue au maintien d'une cote de saveur plus élevée dans les tests sensoriels.

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INTRODUCTION of mechanical flesh-separating machines in the fish-processing industry has significantly increased the utilization of several previously difficult-to-market fish species. In the freshwater fishery, species of the family Catostomidae, suckers, known commercially as mullet, have had little market success partly because of the distribution of fine intramuscular bones in the fillet. However, utilizing the bone-free mince has now given mullet a degree of market acceptance. A major problem encountered with this method of processing is the poor storage characteristics of the frozen mince.

There has been little work in respect to changes that occur in stored minced fish muscle, particularly freshwater fish. Grieg et al. (1967) showed that of propyl gallate, monosodium glutamate, sodium tripolyphosphate, and ascorbic acid, only ascorbic acid extended the shelf life of frozen minced cisco (lake herring). There has been no reported work on the effects of antioxidants on the storage stability of frozen minced sucker.

An accelerated storage test on frozen minced sucker was undertaken by adding singly and in combination a commercial antioxidant, a chelat-

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TABLE 1. Treatments applied to minced sucker for storage test; (dry chemicals were dissolved in water prior to adding to the minced sucker).

| Treatment Number | Water[a] % | Disodium[a,b,c] EDTA | Sodium[a,c,d] ERY | Sodium[a,c,e] TPP |
|---|---|---|---|---|
| 1 (ERY, TPP) | 5 | — | 0.1 | 0.5 |
| 2 (ERY) | 5 | — | 0.1 | — |
| 3 (EDTA, TPP) | 5 | 0.025 | — | 0.5 |
| 4 (EDTA) | 5 | 0.025 | — | — |
| 5 (ERY, EDTA, TTP) | 5 | 0.025 | 0.1 | 0.5 |
| 6 (ERY, EDTA) | 5 | 0.025 | 0.1 | — |
| 7 (TPP) | 5 | — | — | 0.5 |
| 8 (Control) | 5 | — | — | — |

[a]Added as a percent of weight of the fish.
[b]Disodium (ethylenedinitrilo) tetraacetate (dihydrate), "Baker" analyzed reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.
[c]Levels used comply with Canadian Food and Drug Regulations and contributed no detectable flavors.
[d]Neo-Cebitate-R.S., Merck Chemical Division, Merck and Co., Rahway, N.J.
[e]FOS-STP Granular (Food Grade Phosphate), The Griffith Laboratories Ltd., Scarborough, Ontario.

ing agent to reduce trace metal-induced oxidative rancidity, and tripolyphosphate to reduce thaw-drip.

*Materials and methods* — Common white and shorthead redhorse suckers (*Catostomus commersoni* and *Moxostoma macrolepidotum*, respectively) were gillnetted at Lynch's Point, Lake Manitoba. The round fish were packed in ice and transported on the same day to the Freshwater Institute where they were headed and eviscerated, washed thoroughly to remove kidney, blood, and slime, iced and stored at −4°C. The fish were held at −4°C for 5 days (the approximate time between harvesting and secondary processing in a commercial situation) and re-iced every 36 h. At the end of this aging period fish were split longitudinally, washed, and the flesh separated by a Baader 694 flesh-separating machine equipped with a separating drum having 5 mm diam perforations.

The antioxidant used, sodium erythorbate (ERY), is the salt of the D-isomer of ascorbic acid. Its chemical reactivity is almost identical to ascorbic acid, its cost is lower, and it is more readily available (Anon. 1976). The metal chelator, disodium ethylenediaminetetraacetate (EDTA), was selected because its usage is currently permitted in fish products to inhibit the development of trace metal-induced oxidative rancidity (Farragut 1972; Power et al. 1968). Sodium tripolyphosphate (TPP) was added because of its commercial application in frozen minced fish to control thaw-drip.

The minced flesh was weighed into 8 × 6-kg lots and treated as listed in Table 1. Salts were dissolved in water to ensure uniform dispersion in the minced fish and blended thoroughly for 30 s in a Hobart LP-800 food mixer at medium speed. Each treatment was divided into 24 250-g samples which were plate-frozen in 12.5 × 10 × 2.5-cm blocks with a polyethylene overwrap to −34°C. Samples were transferred to a −34°C jacketed cold storage room for 24 h to ensure the absence of temperature variation at the start of the storage test. Also, initially, a sample of untreated minced flesh was withdrawn, packed into 50, 307 × 200.5 cans (standard industry designation meaning $3\frac{7}{16}$ in. diam by $2\frac{1}{36}$ in. ht for the round cans), vacuum-sealed, frozen, and stored at −51°C to be used as reference samples during sensory testing.

For the storage test all the plate-frozen samples were transferred to a holding room controlled at −4°C. Duplicate samples were withdrawn from each treatment lot beginning at 0-time (when samples were moved from −34 to −4°C). Samples were immediately vacuum sealed in Mylar®–polyethylene laminate and stored at −51°C until chemical and sensory testing was performed. Previous work in this laboratory has shown that samples of minced mullet containing no additives and held under these same conditions remain stable for long periods.

| Time held in storage (mos) | Optical density (TBA value) |
|---|---|
| 0 | 0.0519 |
| 2 | 0.0554 |
| 4 | 0.0483 |
| 8 | 0.0765 |
| 10 | 0.0972 |
| 12 | 0.0745 |

Samples for chemical analysis were removed from frozen storage and placed in a water bath at 20°C. The 2-thiobarbituric acid (TBA) test was used to measure the degree of oxidative rancidity in the minced fish following the method described by Vyncke (1970) using propyl gallate and disodium EDTA in the extract. In preliminary work, the filtrate obtained from minced sucker resulted in a

turbid solution after heating during the color reaction. For this reason, Dr L. C. Dugal and H. Boese of our laboratories modified the method for use with Catostomidae by decreasing the amount of sample used and applying the color reaction procedure described by Tarladgis et al. (1960). The final method used was:

Extraction procedure: to 10 g of fish tissue are added 100 ml of 5% trichloracetic acid solution containing 0.02 g *n*-propyl gallate and 0.02547 g disodium EDTA, blended for 1 min in a Waring blender, and immediately filtered through Whatman No. 1 filter paper.

Color reaction: 5-ml filtrate and 5-ml TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) are placed in a screw-top test tube, capped, and placed in boiling water bath for 35 min. Following 10 min cooling under running water, optical density of each sample was read at 538 nm against a blank containing 5 ml TBA reagent and 5 ml extraction reagent (5% TCA reagent plus *n*-propyl gallate and disodium EDTA) in a Bausch and Lomb Spectronic 20 spectrophotometer. Two replications of the TBA analysis were performed and the average reported.

*Sensory analysis* — An 11 member panel of volunteers experienced in sensory evaluation of fish was selected. Because of the range of flavor attributes that must be considered as samples change during aging, a method of scaling was chosen so that the presence of varying flavor attributes in the samples governed their score rather than the intensity of any one characteristic such as rancidity. A suitable questionnaire was developed using previously aged and frozen minced sucker (Fig. 1). Twenty-four panel sessions were held to evaluate eight randomly selected stored samples per session (96 treatment combinations × 2 duplicates = 192 samples). Eleven judges tasted at each session. Panel sessions were held in a quiet air-conditioned room equipped with individual panel booths and conventional fluorescent lighting. Water, lemon solution (1.6% lemon juice in water), and unsalted crackers were provided as rinsing materials.

For each panel session samples were thawed under running water for 1 h. Each block of minced fish was removed from the package, blended in a tabletop mixer for 10 s, then divided into approximately 10-g portions which were wrapped in 15-cm squares of tinfoil and coded with a three-digit number. Samples were steamed for 8 min and presented on heated trays (Cornwall model 2615). At the conclusion of each session the samples tested were identified to the panelists to allow further learning and reinforcement to occur as panels progressed.

*Data analyses* — Linear regressions were calculated using the sensory and chemical scores against time for each treatment to evaluate the rate of change within each treatment (Steel and Torrie 1960). Using the method described by Brownlee (1960) the lines within each method of testing were compared. Linear

NAME_____ DATE_____

FLAVOR EVALUATION

| DESCRIPTION | SCORE | SAMPLE NUMBERS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| - typical musty, sweet, bland, papery or starchy flavors<br>- no off-flavor | 10 | | | | | | | | |
| | 9 | | | | | | | | |
| - slight musty, sweet, or bland flavors<br>- flat, no off-flavors | 8 | | | | | | | | |
| | 7 | | | | | | | | |
| - slight fishy, sour, or rancid off-flavors<br>- sickly sweetness | 6 | | | | | | | | |
| | 5 | | | | | | | | |
| - definite sour, rancid, or fishy off-flavors<br>- metallic aftertaste | 4 | | | | | | | | |
| | 3 | | | | | | | | |
| - strong off-flavors, very sour or rancid, very fishy<br>- bitter aftertaste | 2 | | | | | | | | |
| | 1 | | | | | | | | |
| texture comment: | tender | | | | | | | | |
| | slt tough | | | | | | | | |
| | ext tough | | | | | | | | |

FIG. 1. Questionnaire used to evaluate flavor changes in minced sucker.

regressions were also calculated to examine the relationship between chemical and sensory scores for each treatment.

*Results and discussion* — The values for slopes (*b*), intercept (*a*), and correlation coefficients (*r*) calculated for the linear regressions of the TBA values vs. the number of days the samples were held in storage and the sensory scores vs. days in storage are given in Tables 2 and 3. In the results of the TBA tests, there is a clear division between effective and noneffective treatments (Fig. 2). Treatments containing ERY, with and without TPP, were all effective in controlling

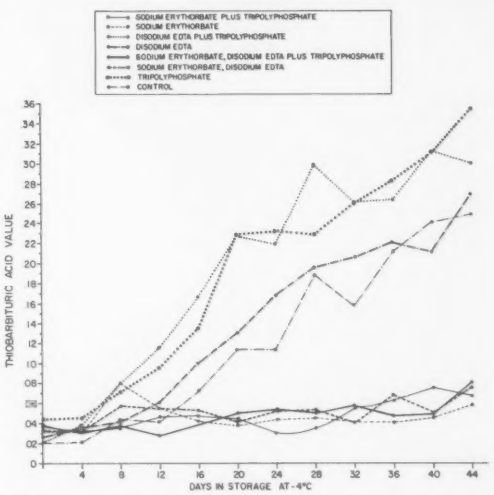


FIG. 2. Changes in levels of oxidative rancidity occurring during accelerated storage tests on minced sucker containing various chemical stabilizers.

TABLE 2. Values from linear regression of TBA values vs. number of days in storage at −4° C.

| Treatment[a] | Intercept ($a$) | Slope ($b$)[b] | $r$ | Student $t$ (regression) |
|---|---|---|---|---|
| 1 (ERY, TPP) | 0.0313 | 0.00077 | 0.76 | 0.67 (n.s.) |
| 2 (ERY) | 0.0455 | 0.00009 | 0.10 | 0.32 ( " ) |
| 3 (EDTA, TPP) | 0.0396 | 0.00696 | 0.95 | 9.91 ($P \leqslant 0.005$) |
| 4 (EDTA) | 0.0133 | 0.00574 | 0.98 | 16.24 ( " ) |
| 5 (ERY, EDTA, TPP) | 0.0308 | 0.00076 | 0.79 | 4.14 ( " ) |
| 6 (EDTA, ERY) | 0.0391 | 0.00058 | 0.63 | 25.87 ( " ) |
| 7 (TPP) | 0.0296 | 0.00737 | 0.98 | 15.81 ( " ) |
| 8 (Control) | 0.0028 | 0.00575 | 0.98 | 14.51 ( " ) |

[a]See Table 1.

[b]Comparing the slopes using the method of Brownlee (1960) resulted in the following groupings:

Treatment No.: 7 3 4 8 (1) 5 6 (2)

TABLE 3. Values from linear regression of sensory values on days in storage.

| Treatment[a] | Intercept ($a$) | Slope ($b$)[b] | $r$ | Student $t$ (regression) |
|---|---|---|---|---|
| 1 (ERY, TPP) | 7.912 | −0.0369 | 0.78 | ($P \leqslant 0.005$) |
| 2 (ERY) | 7.878 | −0.0652 | 0.90 | ( " ) |
| 3 (EDTA, TPP) | 8.542 | −0.1282 | 0.93 | ( " ) |
| 4 (EDTA) | 8.295 | −0.1089 | 0.86 | ( " ) |
| 5 (ERY, EDTA, TPP) | 8.699 | −0.0528 | 0.90 | ( " ) |
| 6 (ERY, EDTA) | 8.513 | −0.0717 | 0.93 | ( " ) |
| 7 (TPP) | 8.379 | −0.1305 | 0.94 | ( " ) |
| 8 (Control) | 7.884 | −0.1212 | 0.88 | ( " ) |

[a]See Table 1.

[b]Comparing the slopes using the method of Brownlee (1960) resulted in the following groupings:

Treatment No.: 7 3 8 4 6 2 5 1

the rate at which oxidative rancidity developed in the minced fish. Here the rate of development of oxidative rancidity was significantly slower when examined using Brownlee's (1960) method to compare the slopes of the regression lines and is approximately 10 times slower than the rate in the ineffective treatments. Correlations are high for the less effective treatments and low for the effective ones. In all probability, this is because daily sample variability in the effective treatments is greater than the variability due to oxidative rancidity.

Oxidative rancidity in the samples was evidently not due to the presence of trace metals since disodium EDTA was ineffective. Thorough cleaning and washing of the fish prior to flesh separation to remove kidney and blood, the probable source of trace metal contaminants, eliminated the need to use a metal-ion chelating agent.

The same groups of effective and less effective treatments are evident in the sensory scores (Fig. 3) even though the differences are not as clearly defined. For all treatments there is a significant regression line that is well correlated to the age of the sample. In following the flavor changes in the samples, the disappearance of the fresh flavor notes occurred whether or not the least change in TBA value maintained the highest flavor scores throughout the sensory testing. Correspondingly, the rate of diminishment of fresh flavor notes was significantly less for the effectively treated samples than for the less effectively treated samples.

Linear regressions of sensory scores vs. TBA values for each treatment (Table 4) show that where the treatments were less effective in controlling oxidative rancidity the correlations are high. Conversely, the effective treatments showed lower or nonexistent correlations indicating that other factors such as textural changes and other flavor changes not measurable as oxidative ranci-

TABLE 4. Linear regressions of sensory scores against TBA values.

| Treatment[a] | Intercept (*a*) | Slope (*b*) | *r* |
|---|---|---|---|
| 1 (ERY, TPP) | 7.86 | −15.87 | 0.34 |
| 2 (ERY) | 6.92 | − 8.08 | 0.09 |
| 3 (EDTA, TPP) | 9.21 | −18.10 | 0.96 |
| 4 (EDTA) | 8.52 | −18.80 | 0.86 |
| 5 (ERY, EDTA, TPP) | 9.68 | −45.31 | 0.74 |
| 6 (ERY, EDTA) | 9.69 | −53.23 | 0.63 |
| 7 (TPP) | 8.83 | −17.34 | 0.93 |
| 8 (Control) | 7.60 | −19.26 | 0.82 |

[a]See Table 1.

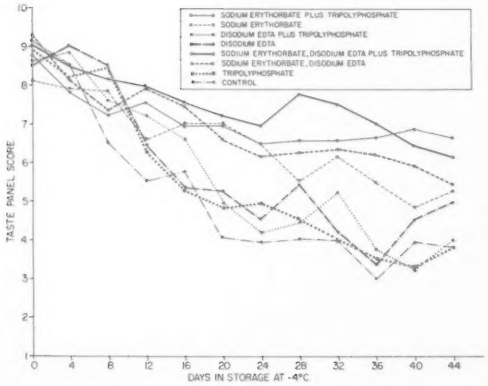


FIG. 3. Changes in taste panel scores occurring during accelerated storage tests on minced sucker containing various chemical stabilizers.

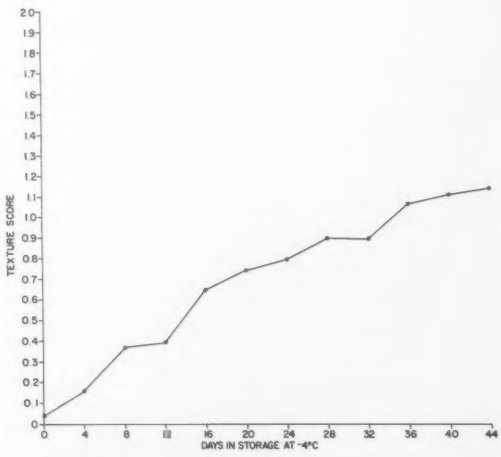


FIG. 4. Mean values of texture scores for minced sucker at each sampling period. 0 = tender, 1 = slightly tough, 2 = extremely tough.

dity in these samples contributed to the panelists' awareness of the age of the samples or their acceptability even when oxidative rancidity was not apparent. Figure 4 illustrates the average change in the texture of the samples with storage time. The mean values for each sampling day were used because the individual treatments showed no difference among treatments in the type of scale used, indicating a progressive toughening of all samples during storage.

An accelerated storage test was used to predict the effect of various chemical treatments on the frozen storage stability of minced sucker. The addition of TPP did not increase or decrease the stability of the samples when used alone or in combination with the antioxidant treatments. Disodium EDTA was not effective in controlling oxidative rancidity when used alone, and when used in combination with ERY, the inhibiting effect was no greater than when ERY was used alone. Sodium erythorbate was the only chemical effective in controlling oxidative rancidity and flavor deterioration in the samples.

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# Relationship Between Two Stock–Recruitment Curves[1]

L. L. EBERHARDT

*Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, Washington 99352, USA*

EBERHARDT, L. L. 1977. Relationship between two stock–recruitment curves. J. Fish. Res. Board Can. 34: 425–428.

The Beverton and Holt and Ricker stock–recruitment curves can be used to generate population growth curves. The Beverton and Holt curve is then identical to a difference equation model for the logistic growth curve, and may be derived in terms of equations for linearly density-dependent population regulation. The same equations lead to the Ricker curve if the density-regulating effect is assumed to depend only on population size at the beginning of the interval between generations. At low rates of population growth, the Ricker curve approaches that of Beverton and Holt. The two curves appear to represent certain concepts known in population biology as "*r* and K selection."

EBERHARDT, L. L. 1977. Relationship between two stock–recruitment curves. J. Fish. Res. Board Can. 34: 425–428.

On peut construire des courbes de croissance de population à partir des courbes stock–recrutement de Beverton et Holt, et de Ricker. La courbe de Beverton et Holt est alors identique à un modèle d'équations de différence applicable à la courbe logistique, et peut être dérivée en termes d'équations pour le contrôle de populations à dépendance linéaire de la densité. Les mêmes équations conduisent à la courbe de Ricker si l'on suppose que l'effet de contrôle de la densité dépend seulement de la taille de la population au début de l'intervalle entre les générations. A de faibles rythmes de croissance de population, la courbe de Ricker se rapproche de celle de Beverton et Holt. Les deux courbes semblent représenter certains concepts connus en biologie des populations comme « sélection *r* et K ».

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WHEN successive generations do not overlap, and the initial stock value is taken near zero, stock–recruitment curves will serve as models for population growth. Such an approach provides a convenient way to illustrate some connections between the two major stock–recruitment curves commonly known as the "Beverton and Holt" and "Ricker" curves. Ricker (1975, appendix III) gives two equations for each curve, and a long list of derived functions for each curve. In common with various other authors, Ricker uses the same symbols ($\alpha$ and $\beta$) for the two parameters of the most frequently used forms of the two curves, and deals with the two curves as separate entities. The purpose of this note is to show how the two sets of parameters may be given a common basis, and that the Beverton and Holt curve can be obtained as a limiting form of Ricker's curve.

*Results* — If the stock or "parental generation" (P) is denoted by $N(t)$ and the recruits (R) or next generation as $N(t + 1)$, then the two stock–recruitment curves may also be regarded as difference equation models for population growth

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curves:

$$R = \alpha P e^{-\beta P} \equiv N(t+1) = \alpha N(t)\, e^{-\beta N(t)} \quad \text{(Ricker)} \quad (1)$$

$$R = \frac{1}{A + \frac{B}{P}} \equiv N(t+1) = \frac{1}{A + \frac{B}{N(t)}} \quad \text{(Beverton and Holt)} \quad (2)$$

Where $N(t)$ denotes population size in the $t^{th}$ distinct generation and $N(t+1)$ represents the next generation.

The Beverton and Holt stock–recruitment curve has origins closely paralleling the logistic growth curve as is evident in its development by those authors (Beverton and Holt 1957: 48). The differential equation for the logistic curve may be written as:

$$\frac{dN}{dt} = r\left[\frac{K - N}{K}\right]N = rN - \frac{r}{K}N^2 \quad (3)$$

where $r$ is usually identified as the rate of increase per unit of time, K is the asymptotic population size, and an initial population size, $N(o)$, is implicitly assumed. The initial course of logistic growth is nearly exponential in character, since the quantity $r/K$ is ordinarily a very small number and thus has little effect when N is small. One can thus speak of $r$ as the "density-independent" parameter and $r/K$ as the "density-dependent" component of population growth (another approach is given below).

The solution of equation (3) is then:

$$N(t) = \frac{K}{1 + Ce^{-rt}} \quad \text{where } C = \frac{K - N(o)}{N(o)} \quad (4)$$

To show that this growth curve is equivalent to the Beverton and Holt stock–recruitment curve, one can write equation (4) for $N(t+1)$ and rearrange the right-hand side. A more compact result is obtained by regarding $N(t)$ as the initial condition and writing down the result for one unit of time:

$$N(t+1) = \frac{K}{1 + \left[\frac{K - N(t)}{N(t)}\right]e^{-r}}$$

$$= \frac{1}{\frac{1 - e^{-r}}{K} + \frac{e^{-r}}{N(t)}} = \frac{1}{A + \frac{B}{N(t)}} \quad (5)$$

Hence the constants of the Beverton and Holt curve are $A = (1 - e^{-r})/K$ and $B = e^{-r}$. This difference equation form of the logistic growth curve was used by Leslie (1958), in a study of the effect of time lags on population growth.

The logistic growth curve is based on continuous mathematics, and the difference equation result above (equation 5) can be regarded as a statement of the relation between the population sizes at uniformly spaced points in time (generations). The underlying model is thus one in which the density-dependent effects operate continuously over the time interval between observations. An alternative model is one in which the population grows exponentially over the time interval between observation points (generations), but at a rate set at the beginning of each such interval. This can be expressed as:

$$N(t+1) = N(t)e^{r(t)} \quad (6)$$

where $r(t)$ represents the rate of growth of the population at time $t$. If this rate is taken to be that of the logistic curve (equation 3 above), then:

$$N(t+1) = N(t)e^{r/K[K - N(t)]}$$
$$= e^{r}\, N(t)e^{-(r/K)N(t)}$$
$$= \alpha N(t)e^{-\beta N(t)} \quad (7)$$

Consequently the constants of the Ricker stock–recruitment curve are $\alpha = e^{r}$ and $\beta = r/K$ when expressed in terms of the parameters of the logistic growth curve.

The above results provide a convenient way to interpret the two stock–recruitment curves in terms of common parameters. Since both curves are here represented as analogs of continuous forms, using the same parameters, it is intuitively apparent that a limiting relationship should hold between the curves. Such a relationship may be shown by writing equation (6) in terms of a continuously variable time interval ($\tau$):

$$N(t+\tau) = N(t)e^{r(t)\tau} = N(t)e^{r/K[K - N(t)]\tau}$$

subtracting $N(t)$ from both sides and dividing both sides by $\tau$:

$$\frac{N(t+\tau) - N(t)}{\tau} = \frac{N(t)}{\tau}\left[e^{r/K[K - N(t)]\tau} - 1\right]$$

The exponental term may be replaced by a series expansion:

$$\frac{N(t+\tau) - N(t)}{\tau} = N(t)\left\{\frac{r}{K}[K - N(t)] + \left(\frac{r}{K}\right)^2 \frac{[K - N(t)]^2\tau}{2!} + \cdots\right\}$$

Then, by the definition of a derivitive:

$$\frac{dN}{dt} = \lim_{\tau \to 0} \frac{N(t+\tau) - N(t)}{\tau}$$
$$= \frac{r}{K}[K - N(t)]N(t)$$

so that Ricker's curve approaches that of Beverton and Holt if the time interval becomes sufficiently small.

A similar result may be obtained by expanding the right-hand sides of equations (1) and (2) and comparing terms. Such a comparison makes it evident that the "closeness" of the two curves depends on the magnitude of $r$. However, the same outcome is intuitively evident if one compares the two sets of parameters. Evidently $\alpha (= e^r)$ of Ricker's curve is identical to 1/B in the Beverton and Holt curve, but $\beta (= r/K)$ is only approximately equivalent to A, with the approximation being very close for small $r (e^{-r} - 1 = r - r^2/2! + r^3/3! - \ldots)$. Even when the rate of growth is large enough to yield population doubling in a generation, the two curves are not so greatly dissimilar as growth curves (Fig. 1) and have much the same left-hand limb as stock–recruitment curves (Fig. 2).

*Discussion* — The results presented here may serve two somewhat similar purposes. One is to describe the two curves by using identical parameters. The long list of related and derived functions given by Ricker (1975, Appendix III) can thus be expressed in terms of the same constants. Whether $r$ and K, two of the constants ordinarily used, or some other combination are to be preferred is a matter that needs to be resolved and will undoubtedly depend on convenience, past practice, or needs of the problem at hand.

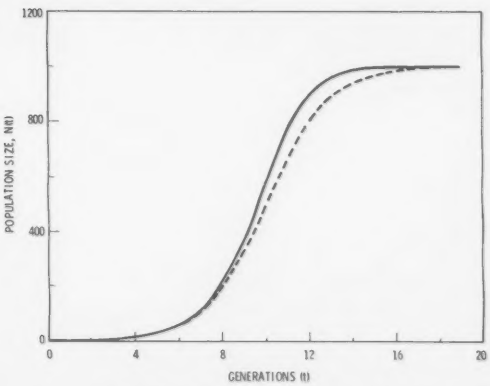


FIG. 1. The Beverton and Holt (dashed line) and Ricker (solid line) stock–recruitment curves expressed as growth curves. Initial population taken as unity, $r = .69315$, K = 1000.

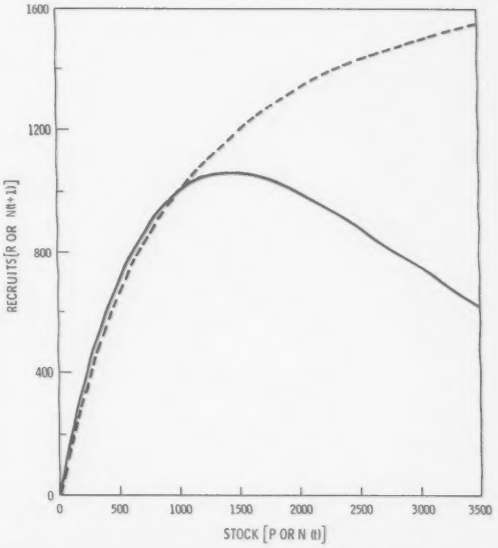


FIG. 2. The Beverton and Holt (dashed line) and Ricker (solid line) stock–recruitment curves resulting from the same basic parameter values. Parameters as in Fig. 1.

A second purpose may be to attach "density-dependent" and "density-independent" labels to parameters. The logistic curve can be also derived in terms of birth ($b$) and death ($d$) rates that are linear functions of current population size, i.e. one can start with:

$$\frac{dN}{dt} = (b - d)N$$

where $b = b_1 - b_2N$ and $d = d_1 + d_2N$. This is then equivalent to equation (3) with $r = b_1 - d_1$ and $r/K = b_2 + d_2$ (cf. Bartlett 1960, p. 30). Consequently $\alpha$ (Ricker) and B (Beverton and Holt) can be regarded as "density-independent," while $\beta$ (Ricker) is "density-dependent," and A (Beverton and Holt) meets that specification to the order of approximation noted above.

The logistic growth model as used here is not realistic in that many of the populations for which stock–recruitment models are particularly useful (e.g. Pacific salmon) do not continuously increase in size between generations. The model may be regarded as a little less fictitious if it is supposed that the density-dependent processes do operate throughout the several stages between generations. Also, density-dependent effects need not operate on both birth ($b$) and death ($d$)

components; one or the other is sufficient to lead to logistic growth (i.e. either $b_2$ or $d_2$ may be zero).

The above interpretations may be regarded as being mainly a matter of convenience in examining and intercomparing curves. They do not necessarily have any bearing on either fitting or utilizing the curves. The analysis given here does not deal specifically with the influences of level of exploitation on parameters of the curves. Incorporating exploitation will, of course, depend on the particular circumstance in which data for generating the curves is obtained.

The interpretation of the two stock–recruitment curves given here may be of special interest in regard to the respective circumstances in which the curves apply. The Ricker scheme is perhaps most notable in that it can represent situations where the population "overshoots" the asymptote (K) and then oscillates about that level. The magnitude of the oscillations depends on the degree of the "hump" in the curve and the associated steepness of the descending right-hand limb of the curve. The Beverton and Holt curve, being essentially generated from logistic growth, is not capable of "overshooting" (unless a time-lag is introduced) and thus lacks the descending right-hand limb. It is this feature that in practice governs the choice between two curves. From the purely deterministic point of view used here, the right-hand portion of the Beverton and Holt curve (beyond the asymptotic value R = P = 1000 in Fig. 2) is essentially an artifact. It does, however, serve to indicate the course followed by a population that is somehow elevated beyond the asymptote (a smooth return to the asymptotic level).

Possibly the most interesting feature of the present analysis is the evidence that the two curves will be nearly identical if $r$ is sufficiently small. There is thus reason to argue that the Beverton and Holt curve is particularly appropriate for populations that can only attain modest increases from generation to generation. Of course, the logistic growth curve can be fitted to rapidly growing populations, so the main practical distinction remains one of "overshooting" and a descending right limb of the stock–recruitment curve.

The two stock–recruitment curves considered here appear to be representative of what has been called "$r$ and K selection" in one area of population biology. According to this theory, "$r$-selected" populations are capable of rapid growth and are thus selected for circumstances in which marked changes of population level are advantageous. Such situations are those of fluctuating habitat and environmental conditions. Relatively stable habitats are supposed to lead to "K-selection" whereby the populations are conditioned to remain close to the asymptotic level. In a simplistic way, the Ricker-type curves are appropriate to "$r$-selected" species, and the Beverton and Holt curves to "K-selection." It is intriguing that one of the recent explorations of the "$r$ and K selection" theory has arrived at curves having very nearly the shape of the two stock–recruitment curves discussed here (Southwood et al. 1974, figure 2).

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# Survival of Two Bacterial Fish Pathogens (*Aeromonas salmonicida* and the Enteric Redmouth Bacterium) in Ozonated, Chlorinated, and Untreated Waters

GARY A. WEDEMEYER AND NANCY C. NELSON

*U.S. Fish and Wildlife Service, Western Fish Disease Laboratory, Naval Support Activity, Seattle, Wash. 98115, USA*

WEDEMEYER, G. A., AND N. C. NELSON. 1977. Survival of two bacterial fish pathogens (*Aeromonas salmonicida* and the enteric redmouth bacterium) in ozonated, chlorinated, and untreated water. J. Fish. Res. Board Can. 34: 429–432.

Ozone and chlorine inactivation curves were determined in three water types at 20°C for the destruction of the fish pathogens *Aeromonas salmonicida*, the etiologic agent of furunculosis, and the enteric redmouth bacterium (ERM). In phosphate-buffered distilled water, 0.01 mg/ℓ ozone inactivated $10^3$ cells/ml of ERM and *A. salmonicida* in 1/2 and 10 min, respectively. Chlorine at this concentration had little effect on either pathogen and a residual of at least 0.05 mg/ℓ was needed to achieve a complete kill within a 10-min contact time. In soft lake water (30 mg/ℓ as $CaCO_3$) a chlorine residual of 0.1 mg/ℓ rapidly ($\frac{1}{2}$– 1 min) inactivated *A. salmonicida* and ERM but in hard water (120 mg/ℓ) *A. salmonicida* was more resistant and 0.2 mg/ℓ chlorine was required. Ozonation of the two lake waters at 90 mg $O_3 \cdot h^{-1} \cdot \ell^{-1}$ (equivalent to a 0.01 mg/ℓ residual in ozone demand-free water) was required to destroy both pathogens within 10 min.

In untreated soft lake water $10^3$ cells/ml of *A. salmonicida* survived only 2 days, while the ERM bacterium ($10^3$ cells/ml) survived even after 20 days in soft and hard untreated lake waters.

WEDEMEYER, G. A., AND N. C. NELSON. 1977. Survival of two bacterial fish pathogens (*Aeromonas salmonicida* and the enteric redmouth bacterium) in ozonated, chlorinated, and untreated water. J. Fish. Res. Board Can. 34: 429–432.

Nous avons déterminé dans trois types d'eau à 20°C les courbes d'inactivation de l'ozone et du chlore pour la destruction des organismes pathogènes des poissons, *Aeromonas salmonicida*, l'agent responsable de la furonculose, et la bactérie de la bouche rouge entérique (ERM). Dans de l'eau distillée tamponnée de phosphate, 0.01 mg/ℓ d'ozone rend inactives $10^3$ cellules/ml de ERM et de *A. salmonicida* en $\frac{1}{2}$ et 10 min respectivement. Le chlore à cette concentration a peu d'effet sur l'un ou l'autre de ces agents pathogènes, et il faut un résidu d'au moins 0.05 mg/ℓ afin d'obtenir une mortalité totale dans les limites d'un contact de 10 min. Dans de l'eau de lac douce (30 mg/ℓ sous forme de $CaCO_3$), un résidu de chlore de 0.1 mg/ℓ rend rapidement ($\frac{1}{2}$–1 min) inactifs *A. salmonicida* et ERM, mais dans de l'eau dure (120 mg/ℓ), *A. salmonicida* est plus résistant et requiert 0.2 mg/ℓ de chlore. Pour détruire les deux agents pathogènes en dedans de 10 min, il a fallu introduire de l'ozone dans les deux eaux de lac au taux de 90 mg $O_3 \cdot h^{-1} \cdot \ell^{-1}$ (équivalant à un résidu de 0.01 mg/ℓ dans une eau libre de demande en ozone).

Dans de l'eau de lac douce non traitée, $10^3$ cellules/ml de *A. salmonicida* ne survécurent que 2 jours, alors que la bactérie de l'ERM ($10^3$ cellules/ml) survécurent même à 20 jours dans des eaux de lac non traitées douces aussi bien que dures.

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OZONE has recently attracted attention for its potential as a disease control agent in intensive fish culture (Rosenlund 1974; Conrad et al. 1975) because improved methods for destroying fish pathogens in hatchery water supplies are needed in at least three phases of fishery practice. Disinfecting hatchery influent water would be desirable in certain instances to permit use of water sources that are presently unsuitable because of enzootic fish diseases. Also, positive disease control in reuse hatcheries through more efficient disinfection of all, or a part of, the recirculated water is a necessity for efficient fish culture. Finally, disinfection of effluent water from fish-holding facilities, preferably with a nonpersistent agent, may be required to prevent discharge of exotic fish pathogens into receiving waters.

Although ozone shows considerable promise as a candidate disinfectant to fulfill these requirements, previous work has been limited almost exclusively to human pathogens (Harris 1972; Broadwater et al. 1973). A study by Burleson et al. (1975) did include one fish pathogen *Pseudomonas fluorescens* suspended in secondary sewage treatment effluent at about $10^7$ cells/ml. It was rapidly (1–2 min) destroyed by relatively low, but variable, ozone concentrations. Conrad

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et al. (1975) showed that ozone effectively destroyed *Flexibacter columnaris* at $10^3$–$10^5$ cells/ml in spring water but again, actual dose and contact time could not be determined.

The present study was carried out to determine ozone inactivation curves for two additional fish pathogens, the enteric redmouth bacterium (ERM) and *Aeromonas salmonicida*, the etiologic agent of furunculosis. Ozone demand-free distilled water and two natural lake waters were used. For comparison, and because little experimental data could be found in the literature, chlorine inactivation curves and survival curves in untreated water were also determined.

*Materials and methods* — Batch disinfection tests at constant ozone or chlorine concentration were carried out at 20°C in an insulated glass contact chamber modified from Majumdar et al. (1973). A PCI (Pollution Control Industries; West Caldwell, New Jersey) model C2P-3C ozone generator was used, fed with oil-free compressed air at a dew point of −40°C. In the ozone demand-free water, residual ozone levels were determined by the method of Shechter (1973). In the two lake waters, a stable residual was difficult to develop as is characteristic of ozone, even after extensive pre-ozonization. In these experiments, it was necessary to express the ozone dose during a run as milligrams of $O_3$ per hour per litre delivered into the water. This rate was determined by exposing 1 $\ell$ of 2% KI in the contact chamber to the desired generator output and titrating the resulting iodine with standardized sodium thiosulfate. A blank run to account for air oxidation was necessary for each calibration run.

For the chlorine tests, sodium hypochlorite was added to achieve the desired residual. At the bacterial cell mass and water volumes used, the residual did not change significantly during the tests. Chlorine was determined by the standard *o*-tolidine method.

Stock ERM and *A. salmonicida* cultures, of confirmed virulence and identity, were stored under oil in Trypticase® Soy Broth at 4°C, and a Trypticase® Soy Agar (TSA) plate was inoculated 1 day prior to each test. The 24-h growth was suspended in sterile phosphate-buffered saline, washed twice, and adjusted to $10^9$ cells/ml using the optical density at 640 nm. Because osmotic shock alone destroyed ERM and *A. salmonicida* when they were suspended directly in ozone demand-free distilled water, it was necessary to add 0.007 M phosphate buffer (pH 7) to stabilize survival so that disinfection tests could be conducted. The two natural lake waters did not require electrolyte additions.

For each test, 1.0 $\ell$ of autoclaved test water, adjusted to 20°C, was added to the contact chamber. Air flow through the gas sparger at the bottom of the column was set at 750 ml/min to assure mixing. Depending on the test, sodium hypochlorite was added or the ozone flow through the capillary was adjusted such that the desired residual developed and stabilized. At time 0, 1.0 ml of the desired bacterial suspension was injected into the column through a sample port to achieve a cell suspension of about $10^3$/ml. A separate 1.0-ml aliquot was taken for a duplicate plate count to determine the actual initial cell count in the column. At exposure times of ½, 1, 2, 5, 10, 20, and 30 min, additional 1.0-ml samples were withdrawn for duplicate plate counts and a 10.0-ml sample withdrawn for an ozone or chlorine determination. Each sample was added aseptically to a dilution tube containing 1-ml phosphate-buffered saline and enough sodium thiosulfate to inactivate 10 times the calculated maximum ozone or chlorine remaining. Samples were diluted out to $10^4$ in sterile, phosphate-buffered saline, and duplicate Trypticase® Soy Agar pour plates prepared from each. Colonies per plate were recorded after 48 h of incubation at room temperature. Counts of less than 30 per plate were recorded as $< 0$ on the log scale used for the figures. Plotted values represent means of duplicated experiments.

To allow for any effect on bacterial survival of the air flow used for mixing, control runs were made with the ozone generator switched off.

*Results and discussion* — Under laboratory conditions in ozone demand-free phosphate-buffered distilled water, the ERM bacterium was considerably more sensitive to ozone than was *A. salmonicida* (Fig. 1A). An ozone residual of 0.01 mg/$\ell$ caused complete inactivation of ERM within 30 s but 10 min was required to inactivate *A. salmonicida*. To achieve a complete kill of *A. salmonicida* with a 30-s contact time required increasing the $O_3$ residual to 0.04 mg/$\ell$. In comparison, 0.01 mg/$\ell$ chlorine for up to 10 min under these conditions did not significantly affect either fish pathogen (Fig. 1B). Increasing the chlorine residual to 0.05 mg/$\ell$ inactivated ERM within 30 s, but only a 2 log reduction of *A. salmonicida* was obtained, even after 10 min of exposure. Complete inactivation of *A. salmonicida* within 30 s required 0.1 mg/$\ell$ chlorine.

The disinfection results obtained in soft (30 mg/$\ell$ as $CaCO_3$) and hard (120 mg/$\ell$) lake waters illustrate the effect water chemistry can have on the amount of ozone needed to destroy fish pathogens (Fig. 2). In contrast to breakpoint chlorination, stable ozone residuals are difficult to achieve in most natural waters. Thus, the following results are expressed as milligrams $O_3$ per hour per litre delivered into the water. In the case of *A. salmonicida*, the hard lake water was somewhat more difficult to disinfect at the lower ozone dose rates of 20 $mg \cdot h^{-1} \cdot \ell^{-1}$ unless the contact time was extended to 30 min (Fig. 2A). Increasing the ozone generation rate to 90 $mg \cdot h^{-1} \cdot \ell^{-1}$ reduced the contact time to a more practical 5–10 min. Similar ozone dose and contact time results were obtained with the ERM

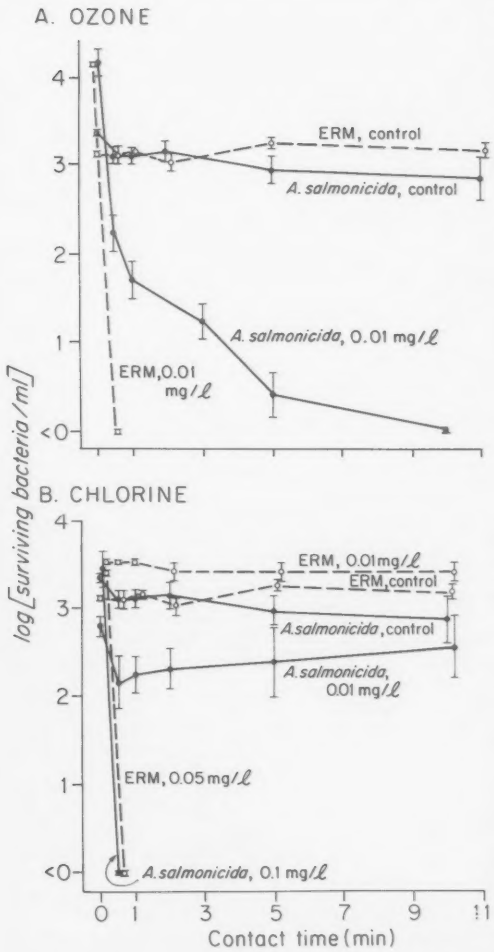


FIG. 1. Ozone and chlorine dose and contact times needed to inactivate *Aeromonas salmonicida* and the enteric redmouth bacterium (ERM) under laboratory conditions in phosphate-buffered distilled deionized water, pH 7, 20°C. Data points are shown as means ± SE.

bacterium (Fig. 2B). A generation rate of 90 mg $O_3 \cdot h^{-1} \cdot \ell^{-1}$ completely disinfected soft and hard lake waters in 10 min.

The ozone disinfection regrowth phenomenon (Venosa 1972), in which biochemical repair of ozone-induced cell wall damage occurs, was not detected in this study. The TSA plates were incubated at room temperature for up to 2 wk and showed no increase in cell counts of either ERM or *A. salmonicida* after partial or complete ozone or chlorine disinfection.

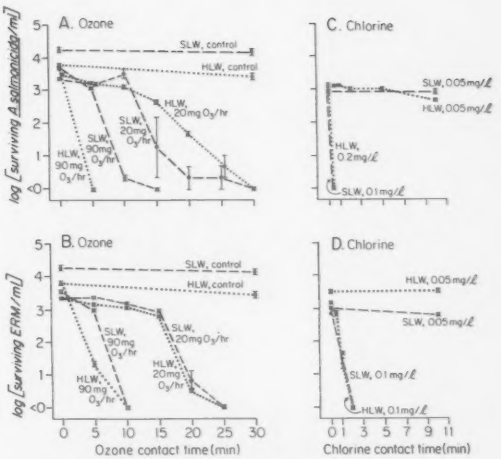


FIG. 2. Ozone generation rates and chlorine residual needed to inactivate *A. salmonicida* and ERM in soft (SLW) and hard (HLW) lake waters as a function of contact time, 20°C. Ozone residuals could not be reliably developed. Data points are shown as means ± SE.

The amount of chlorine needed to destroy these two fish pathogens was also affected by water types but, in contrast to ozone, a stable residual could be obtained (Fig. 2C, D). A chlorine level of 0.1 mg/ℓ destroyed *A. salmonicida* suspended in soft lake water within 30 s (Fig. 2C). In the hard lake water increasing the level to 0.2 mg/ℓ was necessary to rapidly inactivate this fish pathogen (Fig. 2C).

In the case of the ERM bacterium, only 0.1 mg/ℓ chlorine was needed for rapid (1–2 min) inactivation in both water types (Fig. 2D). This level of chlorine had essentially no effect on *A. salmonicida* in hard lake water (Fig. 2C).

The survival time of pathogens in water supplies that are left untreated is also important in fish disease control. Results at 20°C for ERM and *A. salmonicida* are summarized in Fig. 3. Again, water type was very important. In deionized water, *A. salmonicida* and ERM died out within 1–2 days, presumably because of osmotic shock, and even in soft lake water, *A. salmonicida* survived only about 2 days. However, survival of *A. salmonicida* in hard lake water was much better, about 2 wk (Fig. 3A). The ERM bacterium survived well in both lake waters. Growth occurred for about 10 days and the plate counts then remained relatively constant until the experiments were terminated after 20 days (Fig. 3B).

Disinfecting soft hatchery water supplies where

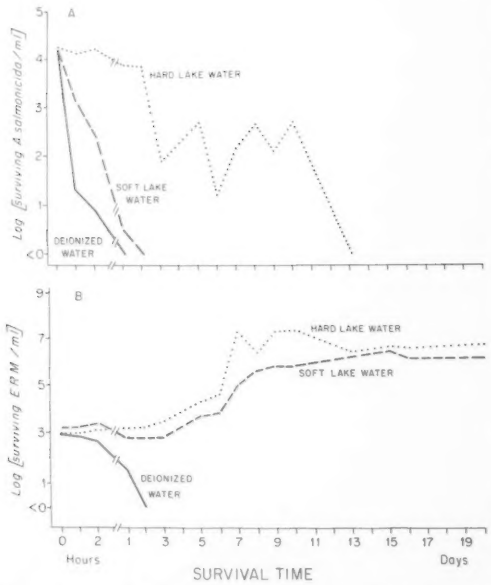


FIG. 3. Survival of *A. salmonicida* and ERM in untreated distilled, soft or hard lake water, 20°C.

furunculosis is enzootic might not be necessary if the reservoir of infection is sufficiently far removed from the hatchery intake to allow a 1–2 day detention time. For enteric redmouth disease control, however, natural die-off within a practical time frame would not be likely even in soft water and treatment would be necessary to remove this pathogen.

Due to the thermodynamic instability of the $O_3$ molecule, stable ozone residuals are more difficult to achieve than are chlorine residuals except under controlled laboratory conditions. However, this instability does not affect disinfection power, and ozone was about twice as effective as chlorine in destroying these two fish pathogens.

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# Therapeutic Treatment for Epibiotic Fouling on Dungeness Crab (*Cancer magister*) Larvae Reared in the Laboratory

WILLIAM S. FISHER AND RICHARD T. NELSON

*University of California, Bodega Marine Laboratory, Bodega Bay, Calif. 94923, USA*

FISHER, W. S., AND R. T. NELSON. 1977. Therapeutic treatment for epibiotic fouling on Dungeness crab (*Cancer magister*) larvae reared in the laboratory. J. Fish. Res. Board Can. 34: 432–436.

This study shows that larvae of the Dungeness crab (*Cancer magister* Dana) reared in the laboratory are susceptible to epibiotic microbial infestation similar to that described for eggs of the same species. Larval survival in the laboratory was increased by increased additions of

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antibiotics to the sea water; however, the chemotherapeutic agent, malachite green, was unsuccessful due to its toxicity to the sensitive larval stages. The addition of the diatom *Phaeodactylum tricornutum* was beneficial to larval survival in the light but detrimental in dark conditions, supporting the suggestion of a photosynthetic excretion from the algae capable of antibiotic activity. Chronic antibiotic treatment was observed to reduce the size of the larvae. Demonstration of larval susceptibility to epibiotic microbial infestation in the laboratory coupled with its occurrence on egg stages in nature provides reasonable evidence that the disease can occur on larval stages in nature.

FISHER, W. S., AND R. T. NELSON. 1977. Therapeutic treatment for epibiotic fouling on Dungeness crab (*Cancer magister*) larvae reared in the laboratory. J. Fish. Res. Board Can. 34: 432–436.

Cette étude démontre que les larves du dormeur du Pacifique (*Cancer magister* Dana) élevées en laboratoire sont sujettes à une infestation microbienne épibiotique semblable à celle décrite pour les œufs de la même espèce. La survie des larves en laboratoire est favorisée par addition d'antibiotiques à l'eau de mer; cependant, l'agent chimiothérapeutique, le vert de malachite, n'a pas de succès à cause de sa toxicité vis-à-vis des stades larvaires sensibles. L'addition de la diatomée *Phaeodactylum tricornutum* favorise la survie des larves à la clarté, mais lui nuit à la noirceur, ce qui confirme la suggestion d'une excrétion photosynthétique de l'algue, capable d'activité antibiotique. Un traitement antibiotique chronique a pour effet de réduire la taille des larves. La démonstration en laboratoire de la susceptibilité des larves à une infestation microbienne épibiotique, couplée à sa présence dans la nature sur les œufs à divers stades est une preuve raisonnable que la maladie peut se rencontrer chez les stades larvaires dans la nature.

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THE collapse of the Dungeness crab (*Cancer magister* Dana) fishery in central California has prompted research into the early developmental stages of the crab for potential causes (Orcutt et al. 1975). Fisher and Wickham (1976; 1977) found higher mortalities and greater microbial epibiotic infestation of eggs from this region than from any other California region sampled. It was suggested that the disease might also cause mortalities in larval stages of the crab. However, due to the lack of knowledge concerning the dispersal and overall survival of the larval stages (Lough 1976), it is not plausible to make a quantitative assessment of the effect of the disease in nature.

Laboratory studies have shown that crab egg survival was increased by addition of antibiotics or chemotherapeutics to the culture water and decreased by the addition of dissolved nutrients (Fisher 1976). This supported the proposition that mortalities observed in crab egg masses were caused by microbial fouling which was enhanced by poor water quality. The following study is a similar investigation to determine whether microbial fouling can cause mortalities in larval stages of the crab. The effects of antibiotics, malachite green, the diatom *Phaeodactylum tricornutum*, and light and dark rearing conditions are examined.

Poole (1966) found it necessary to use antibiotics while rearing *C. magister* larvae, but other workers have found it sufficient to filter and sterilize the sea water (Reed 1969; Buchanan et al. 1970). Glimme (1974) did not use antibiotics and found bacterial infestation played an important role in the mortality of *C. magister* larvae. Malachite green has been used effectively as a general therapeutic for cultured lobster larvae (Fisher et al. 1976a; 1976b) and as a fungicide on cultured shrimp (Bland et al. 1976). Armstrong et al. (1976) found the chemical unacceptable as a potential treatment for fungal (*Lagenidium*) infections of *C. magister* larvae, since continuous treatment with daily replenishment of the solution was toxic to the larvae at relatively low concentrations (96-h LC50 = 0.04 mg/$\ell$). The addition of phytoplankton may remove metabolites, release oxygen, supply nourishment, and provide bacteriastatic activity (Brick 1974). *Phaeodactylum tricornutum* has been found to contain a soluble organic compound with antibiotic activity which is released during photosynthesis (Chapman and Rae 1969).

*Materials and methods* — Ovigerous Dungeness crabs (*C. magister*) were caught in Auke Bay, Alaska, wrapped in paper toweling, packed in ice, and flown to the Bodega Marine Laboratory, Bodega Bay, Calif. on April 27, 1976. They were held in filtered (1 $\mu$m), sterilized (ultraviolet irradiation) sea water at ambient temperatures (10–12°C) in the laboratory. Larvae hatched from two of the females were used in these experiments.

*Experiment I* — Hatching occurred on May 12, and 2 days later, 50 larvae from a single female were pipetted (wide bore pipette) into each of 18 glass jars (12.5 cm diam × 18 cm high) containing 1.5 ℓ of filtered, sterilized sea water. The jars were covered with transparent lids and placed in a water bath at ambient temperature and a normal (about 14h:10h) light:dark regime. All glass jars were inoculated to a final density of 25,000/ml with the algae *Skeletonema costata* as a food source. The larvae were observed and counted, the water was exchanged, and the algae were replaced 3 times weekly. Duplicate jars were treated with 10–100 mg/ℓ of both streptomycin and penicillin-G (1670 U/mg) or 0.001–1.0 mg/ℓ malachite green with each water change. Control groups were held without any treatment. *Artemia salina* nauplii were substituted on day 19 as the food source. They were fed to larvae 3 times weekly to a final concentration of 3 nauplii/ml. The experiment was terminated on day 40.

*Experiment II* — Eggs from a second female hatched in the same seawater system on June 4. After 2 days, 15 larvae were pipetted into each of 18 600-ml glass beakers with glass lids, each holding 500 ml of filtered and sterilized sea water, and placed in a water bath at ambient temperature. The culture water was exchanged 3 times weekly, at which times the larvae were counted and fed *Thallasiosira pseudonana* to a final density of 25,000/ml (the algae fed in Experiment I (*S. costata*) were not available). Duplicate beakers were used for the various treatments. In normal and dark regimes antibiotics and/or *P. tricornutum* were added with the water changes. Streptomycin and penicillin-G (1670 U penicillin-G/mg) were added to a final concentration of 100 mg/ℓ each and *P. tricornutum* were added to a final density of 25,000/ml (in addition to the *T. pseudonana* used as food). The feeding regime was altered on one set of duplicate beakers held in the dark with antibiotics. In this case, *A. salina* nauplii at a density of 3 naplii/ml were substituted for the algal diet (*T. pseudonana*) on day 7. The success of this particular case prompted the decision to substitute *A. salina* nauplii for *T. pseudonana* on day 14 (rather than on day 19 as in Experiment I) for the remaining beakers in the experiment. The experiment was terminated 21 days after eggs hatched.

*Results — Experiment I* — All larvae treated with antibiotics showed higher survival than the untreated controls, and increasing concentrations of antibiotics increased survival (Fig. 1). Malachite green increased survival very slightly at the lowest concentration (0.001 mg/ℓ), but increasing concentrations decreased crab survival (Fig. 2) such that all animals in 1 mg/ℓ malachite green were dead by day 6. The untreated larvae showed moderate to heavy microbial infestation while those treated with 100 mg/ℓ antibiotics showed only slight infestation. The types of epibionts observed were similar to those described

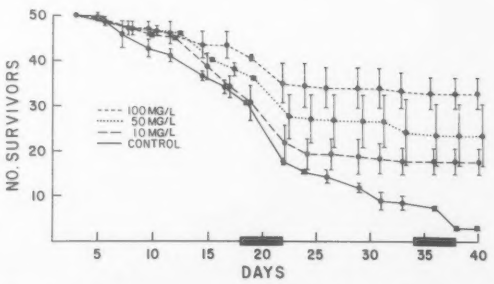


FIG. 1. Results from Experiment I of antibiotic treatment on crab larvae using streptomycin and penicillin-G (1670 U/mg); controls were untreated. Increased concentrations of antibiotics increased larval survival. The shaded areas (days 18–22 and days 34–38) represent periods of molting to second and third zoeal stages.

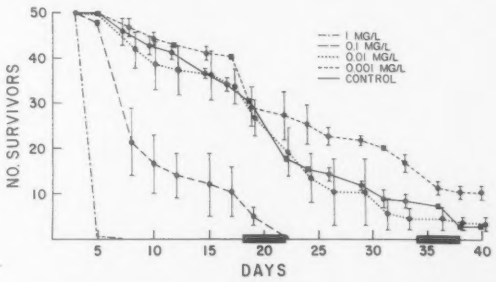


FIG. 2. Results from Experiment I of malachite green treatment on larval survival showing some success of low levels but becoming toxic with increased concentration. Controls were untreated. Shaded areas represent periods of molting to second and third zoeal stages.

by Nilson et al. (1975), and the predominant type was similar to the cyanophyte *Oscillatoria*. The treated larvae were observed to be smaller than the untreated, although no measurements were made.

*Experiment II* — Under normal light conditions (Fig. 3) the larvae inoculated with *P. tricornutum* showed slightly higher survival than the untreated controls. Antibiotic treated larvae (Ab and Ab/Pt) had the highest survivals with little difference between them.

In the dark conditions (Fig. 4), larvae treated with *P. tricornutum* showed the poorest survival, all animals dying by day 14. The large disparity shown by duplicate runs of the antibiotic-treated larvae and the untreated control larvae negates comparison between these conditions, although it is clear that antibiotic treatment was beneficial until day 12. Larvae treated with antibiotics and

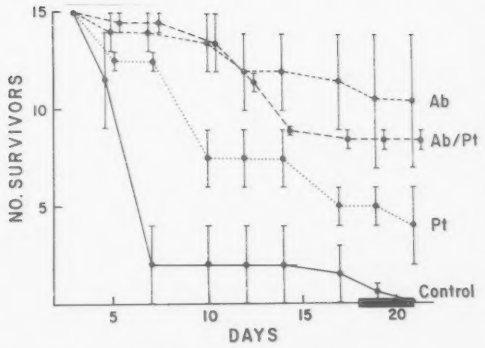


FIG. 3. Results of the normal light regime from Experiment II. Ab — antibiotics; Pt — *P. tricornutum*; Ab/Pt — both. The shaded area shows the molting period to the second zoeal stage.

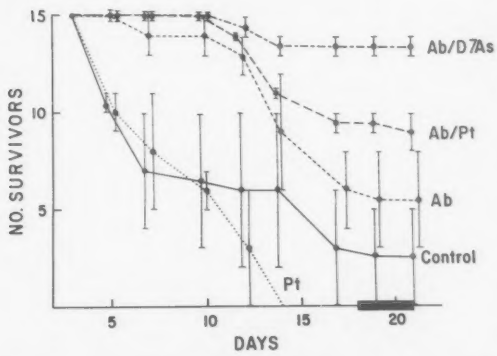


FIG. 4. Results of the dark regime from Experiment II. Ab — antibiotics; Pt — *P. tricornutum*; Ab/Pt — both; Ab/D7As — antibiotics plus *A. salina* on day 7 rather than day 14. The shaded area shows the molting period to the second zoeal stage.

*P. tricornutum* maintained high survival throughout the experiment. The best survival (90%) was shown by the larvae held in the dark with antibiotic treatment and the earlier (day 7) substitution of *A. salina* nauplii.

*Discussion* — The results indicate that *C. magister* larvae are susceptible to epibiotic fouling. The addition of antibiotics reduced microbial infestation and enhanced survival of the larvae. Other workers in central California (Poole 1966; Glimme 1974) have found the lack of antibiotics allows heavy bacterial infestations and causes larval mortalities, while workers in Oregon (Reed 1969; Buchanan et al. 1970) have apparently had no difficulties in similar static systems. It is plausible that these conflicting reports are a result of water quality. Walne (1958) showed that chemical variations create differences in the number of bacteria the water will support when confined in the laboratory.

Malachite green was an effective chronic treatment for epibiotic fouling of Dungeness crab eggs (Fisher 1976), but was unsuccessful on larvae because of its toxic effects. Low concentrations (0.001 mg/$\ell$) appeared to aid slightly in larval survival but the toxicity of increasing concentrations and chronic applications offset the benefits of the treatment. In this study, malachite green solutions were renewed 3 times weekly, while Armstrong et al. (1976) renewed solutions daily to obtain a 96-h LC50 = 0.04 mg/$\ell$. Since malachite green deteriorates in sea water (unpublished results show a 3–5-h half-life at 20°C), the higher survivals maintained in this experiment are not inconsistent with the earlier results.

*Phaeodactylum tricornutum* improved crab survival in the normal lighting regime only, which supports the suggestion of a photosynthetic excretion with antibiotic activity (Chapman and Rae 1969). The detrimental effects in the dark regime were possibly caused by the additional contamination of inactive *P. tricornutum* on the surfaces of the larvae.

It has been shown that chronic treatment with antibiotics increases larval survival, but it also appears to reduce the size of the larvae. Although quantitative experiments are required, this would be consistent with the suggestion of Brick (1974) that antibiotics may eventually become detrimental by acting on symbiotic bacteria in the digestive tracts of the cultured species.

Nauplii of the brine shrimp, *Artemia salina*, have been used successfully as food for the culture of a wide variety of marine decapods (Rice and Williamson 1970) and *C. magister* does not appear to be an exception (Poole 1966; Reed 1969). Glimme (1974), however, felt that these food organisms, although nutritionally adequate, were a probable source of disease microorganisms. Experiment II shows that high survivals may be maintained without *A. salina* nauplii for the first 7 days of rearing (Fig. 4, Ab/D7As).

Egg and larval stages of *C. magister* held in the laboratory show symptoms of a common disease. Epibiotic fouling occurs in both cases and survivals increase when the fouling is retarded. Antibiotics increase survival in both cases, suggesting bacterial responsibility for mortalities, even though other microorganisms are also present. Death may be caused either by restriction of gaseous exchange across egg membranes or larval gill membranes or by the entanglement of larval appendages by filamentous forms (Nilson et al.

1975). This demonstration of the disease in the laboratory combined with its occurrence on egg stages in nature (Fisher and Wickham 1976) lends support to the suggestion that microbial epibionts may be responsible for larval mortality in nature.

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# Detection of Trace Contaminant Effects in Aquatic Ecosystems

SAMUEL N. LUOMA

*U.S. Geological Survey, Menlo Park, Calif. 94025, USA*

LUOMA, S. N. 1977. Detection of trace contaminant effects in aquatic ecosystems. J. Fish. Res. Board Can. 34: 436–439.

If one population of a species is more resistant to a toxicant than are other populations, it is direct evidence that the concentration of the toxicant in the environment of the resistant population is sufficient to elicit biological effects. The presence of a toxicant-resistant population of one species in an ecosystem further suggests that other species may have been affected by the resistance-eliciting substance.

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LUOMA, S. N. 1977. Detection of trace contaminant effects in aquatic ecosystems. J. Fish. Res. Board Can. 34: 436–439.

Si une population d'une espèce est plus résistante à un toxique que d'autres populations, ceci est une preuve directe que la concentration du toxique dans l'environnement de la population résistante suffit à provoquer des effets biologiques. La présence d'une population résistante à un toxique d'une espèce dans un écosystème suggère de plus que d'autres espèces peuvent avoir été affectées par la substance provoquant cette résistance.

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DIRECT evidence that trace contaminants adversely affect natural populations of aquatic organisms is relatively scarce. Extrapolation of toxicity data to natural systems is limited by the physicochemical complexity of the natural environment and the inherent limitations of toxicity bioassays (Lee 1973). Elevated concentrations of trace contaminants are often observed in ecosystems where the degradation of a biological community is suspected (e.g. Odum et al. 1969; Hodson and Sprague 1975). However, proving that trace contaminants cause the observed effects is difficult, as is separation of the relative biological significance of individual contaminants present. In lieu of more direct methods, I propose application of a well-studied toxicological principle as a means of helping determine i) if trace contaminants are affecting organisms in a given situation, and ii) which contaminants are having the more significant effect.

*Tolerance to toxicants* — The resistance of some insects to both inorganic and organic insecticides is widely documented (Brown 1958; O'Brien 1967). Tolerance to heavy metals has also been studied in terrestrial grasses from polluted areas (McNeilly 1968; Rocovich and West 1975). In both grasses and insects, toxin resistance has similar characteristics: i) resistance results from Darwinian selection for resistant genotypes; it cannot be induced over the lifetime of individuals; ii) physiological mechanisms specific for a single toxicant or group of toxicants are usually involved in resistance, rather than selection for vigorous strains; iii) resistance reduces the overall fitness of the population; when the selective pressure of the toxicant is removed the populations revert, in a few generations, to dominance by intolerant genotypes; and iv) the degree of tolerance is related to the level of exposure to the toxicant.

Resistance to toxicants has also been reported within aquatic species. Resistance to the insecticide endrin occurs in at least six species of fish (Grant 1976). Resistant strains are found only in areas of known contamination; the level of resistance reflects the degree of contamination; and the mechanism of resistance is specific for insecticides. Resistance to cyclodiene insecticides in populations of mosquito fish also occurs in contaminated areas, but is not present where cyclodienes are not present (Ferguson 1970).

Populations of phytoplankton (*Scenedesmus acutiformis* and *Chlorella fusca*) from metal-contaminated lakes near Sudbury, Ontario, are much more resistant to the toxic effects of copper and nickel than populations of these species from uncontaminated areas (Stokes et al. 1973; Stokes 1975). Populations of the polychaete *Nereis diversicolor* from English estuaries contaminated with Cu, Cd, Pb, and Zn are more resistant to Cu than are *N. diversicolor* from less contaminated areas (Bryan and Hummerstone 1971, 1973; Bryan 1974). *Nereis diversicolor* from some, but not all, of the polluted estuaries are also resistant to Zn. No resistance to Cd or Pb was detectable despite high concentrations in sediments. In laboratory studies, resistance to Cu could not be induced within individuals from one generation of *N. diversicolor* collected from an unpolluted estuary.

The range of genotypes expressed in a population from a given location reflects selective pressures against that population. To induce tolerance, a toxicant must be present in biologically available quantities sufficient to limit the reproductive success of a proportion of the individuals in a population (i.e. the nonresistant genotypes). Greater resistance to a toxicant in a population from one location than in a population from a second location is direct evidence that the toxicant is exerting selective pressure on the species at the former site. Moreover, resistance to one toxicant, but not to others to which the population is exposed, suggests that the former contaminant is the cause of the biological damage.

Evidence that a toxicant has exerted selective pressure on one species within a community strongly indicates toxic effects on other species. Many of the organisms in which resistance is

observed (e.g. *Scenedesmus*, *Chlorella*, *Gambusia*) are ecological "opportunists". Such species are characterized by a broader genetic plasticity than species that occupy more specialized niches (Ayala 1968; Grassle and Grassle 1974). The opportunists' ability to survive is enhanced by the variety of genotypes available for colonizing ecologically open space. When a toxicant is introduced into an ecosystem, the probability of an opportunist species developing a resistant population, and surviving, is greater than the probability of a more specialized species exhibiting such resistance. Simplified ecosystems will result when species disappear because they lack contaminant-resistant genotypes. Higher trophic level organisms appear especially susceptible to such effects. Where opportunistic species of fish exhibit resistance to endrin, the number of species in the community is restricted, top carnivores being most commonly absent (Grant 1976). Similarly, predaceous insects are less likely to develop insecticide resistance than are pests (Georghion 1972). The result of insecticide applications is often a simplified insect community, with a reduced capability for internal biological regulation.

*Applications* — Viewing toxicant resistance as an indicator of selective pressure in an ecosystem may provide a new tool for assessing the causes of simplification in aquatic communities. For example, the absence of toxicant sensitive species in a community may be more convincingly attributed to a toxicant if it has induced resistance in a surviving species. Similarly, the resistance of polychaetes to Cu and occasionally Zn, but never Pb or Cd, in estuaries contaminated by all four metals (Bryan 1974) is strong evidence that Cu and Zn are the contaminants of primary concern in these ecosystems. The use of toxicant resistance to relate cause and effect requires comparing toxicity to individuals from a contaminated system with toxicity to individuals of the same species from a less contaminated location. Such comparisons are valid only if individuals of the same life stage are used, and the tests are conducted simultaneously (to avoid temporal effects) under similar physicochemical conditions. Small, random variations in the susceptibility of different populations may make toxicity to highly susceptible genotypes difficult to detect with standard bioassay techniques. However, bioassays can detect general population shifts to resistant genotypes (Ferguson 1970; Bryan 1974; Stokes 1975) and increases in resistance with increasing contaminant concentrations (Grant 1976).

Further study of toxicant resistance in natural systems would increase its usefulness as a tool in environmental studies. An understanding of the community changes that accompany the resistance of surviving species to specific types of toxicants may facilitate cause and effect analyses, and point to toxicant-sensitive species. Comparisons of the biologically available concentrations at which different contaminants induce the onset of resistance would facilitate a better understanding of the relative toxicity of such contaminants in nature. More knowledge about cross-resistance (the development of resistance to a second toxin by exerting pressure with a first) in aquatic species is also necessary. Some cross-resistance to insecticides has been observed in insects (O'Brien 1967), but cross-resistance to trace metals appears to be rare, at least in plants and polychaetes (McNeilly 1968; Bryan 1974).

The use of toxicant resistance to assess contaminant effects has several advantages over direct extrapolation of toxicity data to natural systems. Using in situ population characteristics to indicate effects eliminates the necessity of correlating toxicity and contaminant concentration. While the latter method is preferable where sufficient information is available, attempts at such correlations have been largely unsuccessful due to the physiological and physiochemical complexities of trace contaminant behavior. Similarly, the need to assess such complexities in bioassays is reduced if the tests are used to compare relative susceptibilities rather than absolute levels of toxicity. Direct evidence of toxicant effects may also facilitate natural system studies of the complexities of toxicant behavior.

Determinations of selective pressures on the basis of population characteristics must always be undertaken with caution. However, until contaminant interactions with the total environment are better understood, the view of toxicant resistance as a response to such pressure might have an important place in monitoring, studying, and interpreting the effects of trace contaminants.

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# Holding of Atlantic Mackerel (*Scomber scombrus*) in Refrigerated Sea Water

D. W. LEMON AND L. W. REGIER

*Environment Canada, Fisheries and Marine Service, Halifax Laboratory, Halifax, N.S. B3J 2R3*

LEMON, D. W., AND L. W. REGIER. 1977. Holding of Atlantic mackerel (*Scomber scombrus*) in refrigerated sea water. J. Fish. Res. Board Can. 34: 439–443.

Refrigerated sea water proved to be an improved method of holding Atlantic mackerel (*Scomber scombrus*). The uniform lower temperature and reduction in available oxygen retarded the development of oxidative rancidity. Textural deterioration was also retarded. The sodium uptake from and the potassium loss to the sea water was not excessive, and taste panelists could not consistently identify samples with elevated sodium content. The addition of carbon dioxide to the RSW did not regularly affect the level of spoilage as monitored by the measurement of trimethylamine. The values, however, were low for all holding systems, even after 9 days. The presence of dissolved carbon dioxide in the fish muscle made the fish unacceptable for canning.

LEMON, D. W., AND L. W. REGIER. 1977. Holding of Atlantic mackerel (*Scomber scombrus*) in refrigerated sea water. J. Fish. Res. Board Can. 34: 439–443.

On a constaté que l'emploi d'eau de mer réfrigérée était une meilleure méthode de conservation du maquereau bleu (*Scomber scombrus*). La basse température uniforme et la réduction de l'oxygène disponible retardent le développement de la rancidité par oxydation. La déterioration de la texture est également retardée. L'absorption de sodium, résultant de la perte de potassium à l'eau de mer par le maquereau, n'est pas excessive, et le groupe de dégustation n'a pu identifier uniformément les échantillons contenant plus de sodium. L'addition de gaz carbonique à l'EMR n'affecte pas de façon régulière le degré d'altération tel que mesuré par formation de triméthylamine. Les valeurs, cependant, sont basses pour tous les systèmes de conservation, même après 9 jours. La présence de gaz carbonique dissous dans le muscle du poisson le rend inacceptable pour la mise en conserve.

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THE Canadian Atlantic mackerel (*Scomber scombrus*) fishery has not been strong or profitable. This is related in a large part to the low quality of the product for sale.

Mackerel move up the Nova Scotian coast in May and June. The fish feed and spawn off the coast and in the Gulf of St. Lawrence and then move southward again during July and August. By the end of September most of the mackerel have moved off into deeper water at the edge of the continental shelf where they winter. The prespawning fish, often referred to as "spring mackerel," are leaner than the post spawning fish or "fall mackerel."

In 1974 Canada's Atlantic mackerel catch amounted to less than 5% of the total world catch (15,000 t) (1975, unpublished data), of which less than 30% was processed for human consumption. These values are representative of the last few years. Fresh and frozen mackerel is not a popular food fish across North America, although fresh mackerel is highly prized by many in areas where it is available. The muscle is high in lipid, thus oxidative rancidity is a problem (Ke et al. 1975). The flesh is fairly delicate so textural deterioration of stored fish is common. These problems are compounded by the fact that fishermen receive a low return for their catch and thus tend to handle and store the fish poorly.

As part of a program designed to assist in the more effective utilization of these fish, studies on the use of refrigerated sea water (RSW) to hold mackerel prior to processing were undertaken. It was expected that this technique would improve the quality of raw material available to the processors and thus improve the quality of products offered to consumers. Refrigerated sea water has been shown to be a superior method of holding fish. Freshly iced fish cool slowly and unevenly because most of the fish are not in contact with ice, thus cooling is accomplished by contact with other cooler fish and by melt water draining through the catch. Under good icing conditions the fish usually equilibrate at 2–3°C, but, this temperature is not uniformly maintained throughout the system. In RSW systems, the sea water, which is mechanically cooled completely surrounds the fish, thus initial cooling is rapid and temperature is maintained uniformly throughout the system during a run. The system is closed, therefore oxygen contact is reduced. Bruising and mashing of the catch are also reduced as the fish are suspended in a liquid medium.

*Materials* — Two identical pilot plant refrigerator sea water systems were used. Each unit consisted of a 270-$\ell$ fiberglass insulated tank, a titanium heat exchanger, a refrigeration unit, a temperature controller, and several control devices to insure safe and efficient operation. The water circulating system was designed to produce good upflow and mixing within the holding tank as well as adequate flow through the heat exchanger. A 40-cm$^2$ manifold of 4 cm diam polypropylene pipe into which a number of 0.6-cm holes had been drilled was constructed and connected via 5 cm diam heavy-walled rubber tubing to the circulating pump. The manifold, which could be moved up and down within the tank, was kept slightly below the water line at all times. The water was pumped out of the tank via the manifold, through the heat exchanger, and returned through a number of small inlets in the bottom of the tank.

In one of the two RSW units a hypodermic needle was inserted in the rubber-tubing connection leading to the pump for feeding carbon dioxide into the flowing sea water at a rate of about 1 $\ell$/min. This flow rate assured that the sea water was saturated with the gas. This treatment was reported to be effective in retarding bacterial growth, a major problem with closed RSW systems (Barnett et al. 1971); Longard and Regier 1974; Hiltz et al. unpublished data).

Five experiments were carried out over a 5-mo period beginning in May. Mackerel (*S. scombrus*) were obtained from commercial traps and thus were alive when taken from the water. In less than 2 h of landing, the catch was placed in the RSW units; consequently, no special handling was involved between landing and the start of a test run other than ordinary trucking of the boxed fish to the laboratory. The fish were 30–40 cm long.

*Methods* — Each of the five runs were carried out in the same manner. Before the fish arrived at the laboratory, 70 $\ell$ of filtered sea water were added to each of the two RSW units which were set to operate at −1°C (±0.5°C). After the units reached temperature, 140 kg of mackerel were placed in each tank. This 2:1 ratio of fish to water was maintained throughout each experimental run by removing water every time fish were taken out for testing.

Approximately 90 kg of fish were also kept well iced in another insulated container which had a bottom opening to let the melt water drain away.

Approximately 45 kg of wet fish were filleted at the start of each run. These fillets served as controls for organoleptic evaluation conducted during the course of the run. The fillets were given a quick rinse with running tap water, drained, placed in nylon–saran–polyethylene laminated bags. The bags were evacuated and sealed in a vacuum sealer, quick frozen in a plate freezer at −40°C and stored at −40°C. Total lipid analysis (Bligh and Dyer 1959) was carried out on a composite sample taken from this group.

Six to eight fish (depending on size) were removed from each of the three storage conditions on every working day during a run. The external appearance was noted and the fish were then filleted. The fillets were not skinned. Because the skin is difficult to remove, mackerel fillets are processed industrially with the skins on. Some of the skin-on fillets were evaluated organoleptically for odor, flavor, and tex-

ture by a taste panel comprising at least eight experienced judges (Dyer et al. 1964; Larmond 1970). A hedonic scale with values assigned in the range from 0 to 100 was used. Other skin-on fillets were ground in a food chopper to produce homogeneous composite samples. Quality deterioration of the fish was monitored by the measurement of trimethylamine nitrogen (TMA-N) (Dyer 1959). Direct biological techniques could not be employed due to limited time and manpower. The level of oxidation rancidity was determined by the reaction of thiobarbituric acid (TBA) with carbonyl compounds using a modified version of the procedure outlined by Vynke (1970) and Lemon (1975). Moisture contents were determined by drying 5 g of the composite sample at 70°C under vacuum for 18 h. Ash contents were determined by heating the dried material in a muffle furnace at 550°C for 18 h. The sodium and potassium content of the ashed material was determined by flame emission spectrophotometry (AOAC Official Methods of Analysis 1970).

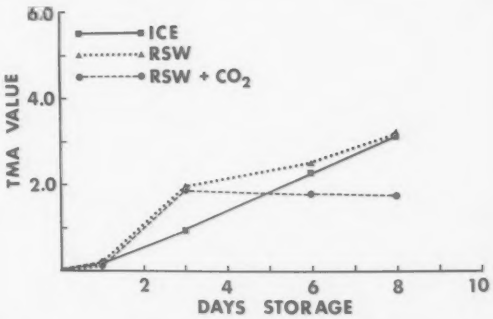


FIG. 1. A representative trimethylamine curve for fillets from Atlantic mackerel (*Scomber scombrus*) stored at −1°C in RSW or crushed ice. The trimethylamine value is expressed as milligrams TMA nitrogen in 100 g of wet fillets.

*Results and discussion* — The most obvious difference between RSW-held and iced fish was in physical appearance and texture. After 3 or 4 days of storage in ice, the fish became soft and some sagging of the belly was evident. By the end of each run the texture was poor, there was noticeable belly sag, the eyes were cloudy, blood was evident around the gills, and strong off-odors were present; however, the iced fish retained the bluish tinge common to mackerel. Despite some softening of the muscle and some slight change in color, the fish held in refrigerated sea water appeared to be in excellent condition. There was no visible difference between the fish held in untreated RSW and those held in RSW saturated with $CO_2$. But both the RSW and RSW–$CO_2$ held fish lost some of the blue coloring, characteristic of mackerel. This was evident, however, only when RSW and iced fish were visually compared to freshly caught mackerel. During two of the runs, Fish Inspection Officers were asked to evaluate the fish after 8 days holding. In both cases the iced fish were immediately rejected. On visual inspection the RSW and RSW–$CO_2$ fish were rated as Grade 1, but the grading was later lowered due to the presence of off-odors.

The $CO_2$ saturated RSW varied in pH from 5.4 to 5.8 while untreated RSW had a pH of 6.4 to 6.7. The effect on the pH of fish musle was minimal. Untreated muscle had a pH of 6.2 to 6.6 while RSW–$CO_2$ fish had a pH of 0.1 to 0.2 U lower.

A definite odor was present in both tanks after 5 or 6 days holding. In all cases, the odor in the untreated RSW tank was more pronounced. This was thought to be indicative of a higher degree of bacterial spoilage in the fish held in untreated RSW. The TMA assays did not bear this out. TMA values from run 2 are plotted against time in Fig. 1. Much the same results were found in other experiments. In three out of four cases the RSW–$CO_2$ fish had slightly lower TMA values than both iced and RSW held fish. For all treatments, though, the TMA values were low, most were less than 5 mg N/100 g. By contrast, previous studies with ocean perch (*Sebastes marinus*) (Longard and Regier 1974) produced values in excess of 25 mg N/100 g in untreated RSW, similarly in experiments on silver hake (*Merluccius bilinearis*) (Hiltz et al. unpublished data) we found 22.6 mg N/100 g after 9 days of untreated RSW holding.

The low TMA values may be partially explained by the fact that mackerel is low in the precursor trimethylamine oxide (TMAO). Dyer (1952) reported TMAO concentrations in mackerel ranging from 31 to 54 mg N/100 g, Mannan et al. (1961), found variations of between 27 and 55 mg N/100 g depending on the part of the muscle sampled, the highest concentration being found in the red muscle. These values are relatively low when compared to the TMAO values of 58–92 mg N/100 g for ocean perch (*S. marinus*) and 89–123 mg N/100 g for silver hake (*M. bilinearis*) reported by Dyer (1952). Yet it is possible that relatively high concentrations of TMA can be found in mackerel. In two separate experiments we kept mackerel for 48 h at approximately 18°C. The TMA concentrations in fillets taken from these fish were 29.5 and 29.8 mg N/100 g. It is therefore plausible that if the holding period had been extended past the 8 or 9 days used in our tests, a marked increase in TMA would also have been seen in the iced and RSW held fish.

TBA values show that oxidative rancidity developed more rapidly in the iced fish than in RSW and RSW–$CO_2$ held mackerel. The TBA curve for run 1 is shown in Fig. 2. This curve is typical of runs 1–4, but not of run 5. In run 5 the control devices that serve to maintain the water temperature at −1°C malfunctioned in the RSW–$CO_2$ system. The temperature rose to 0°C, and at one point touched +1°C. The TBA values for the fish held in this system were higher at 5 and 8 days than would have been predicted from the data compiled during the previous runs. Although one would assume that the level of rancidity should be lower in RSW held fish because of the lesser quantity of oxygen available, especially in the RSW system saturated with $CO_2$, the temperature also plays a role as shown by the results of run 5. In commercial storage the rancidity levels in the iced fish would probably be much higher than those found here, as we used relatively small quantities of fish which were extremely well iced.

In runs 1 and 2, after 9 and 8 days, respectively, the RSW–$CO_2$ fish had statistically better (based on 95% confidence limits) taste panel scores than the iced fish (Fig. 3A, B). On the hedonic scale that we employed, a score of less than 40 corresponds to an organoleptically unacceptable product. Thus, the iced fish in both runs was found to be barely acceptable. This trend continued in run 3, but the taste panel data for this experiment is incomplete and therefore it is not included. The taste panel results were not totally consistent. In run 4 (Fig. 3C) for example, the RSW fish became unacceptable after 8 days, their flavor score being statistically different from the RSW–$CO_2$ and iced fish. This deviation from the trend was perhaps due to an exceptionally high value of TMA after 8 days of storage in RSW. Whereas in Run 5 (Fig. 3D) the flavor scores indicate the RSW–$CO_2$ held fish to be inferior to the iced and RSW samples. This is probably a result of the higher temperatures experienced in that system during the run. The texture scores followed the same trend as the flavor scores, but they were higher and the difference between samples was not as pronounced. Most of the off-odors associated with the mackerel in both RSW systems were romoved on cooking.

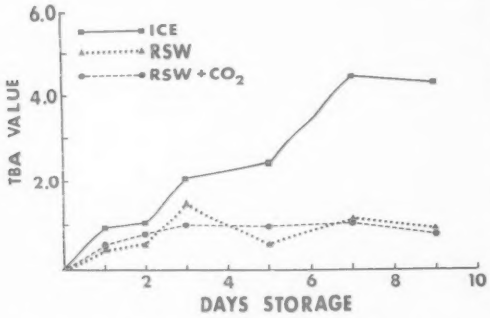


FIG. 2. A representative TBA curve for fillets from mackerel stored at −1°C in RSW or crushed ice. The TBA value is expressed as micromoles of malonaldehyde in 100 g of wet fillets.

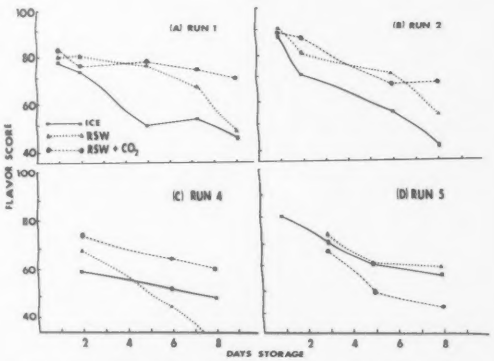


FIG. 3. Taste panel results for fillets from mackerel stored at −1° in RSW or crushed ice.

Sodium uptakes and potassium loss for fillets from fish held in RSW and RSW–$CO_2$ followed the trend observed by Longard and Regier (1974); sodium diffused into and potassium out of the fish muscle. The average of the initial sodium concentrations was about 60 mg/100 g fish (as Na). Others (Harrison and Roach 1953; Longard and Regier 1974) have found that a salt concentration of 250 mg/100 g (as Na) is organoleptically detectable in other species. This was not found to be the case with mackerel. Those species previously studied were rather bland; in fact, some authors have stated that the increased salt concentrations enhanced the flavor. Mackerel has such a distinctive flavor, and is so high in lipid, that the taste panelists were not able to consistently pick out samples with elevated salt levels, even when these levels were well in excess of 250 mg/100 g.

The moisture content of the mackerel did not vary much during holding in RSW and in ice. There was, however, some difference in the moisture contents of spring and fall fish due to the increased amount of lipid in fall mackerel. The average lipid contents of fillets taken during the five experiments were 9.1, 11.8, 15.6, 17.0, and 17.9%. The increased lipid content of the fall fish did not appear to significantly affect keeping quality in the systems under study.

On day 7 of run 4, 23 kg of fish were removed from each holding treatment. The fish were headed and gutted and canned in 0.45-kg tall cans (300 × 407).

The RSW and RSW–$CO_2$ held fish had fairly good texture, but the iced fish were soft and therefore difficult to handle. Two 50-grain salt tablets were added to each can. One half of the lot of cans were precooked in steam at atmospheric pressure for 40 min, then vacuum sealed and retorded for 100 min at 115°C. The other half of the lot was simply vacuum sealed and retorted. Ten cans of each of the six possible combinations were produced.

Precooking revealed that mackerel held in RSW–$CO_2$ contained a great deal of dissolved $CO_2$. Bubbles of escaping gas were visible. A thick "curd" was seen floating in brine. The cooled cans had a slight internal pressure rather than vacuum after vacuum sealing and retorting, meaning that an excess of $CO_2$ was still present in the fish. The cans containing RSW–$CO_2$ mackerel that were not precooked, but simply sealed and retorded, were swollen to the point of being permanently deformed due to excess internal pressure produced by the dissolved $CO_2$. Obviously then, if the fish is to be canned, $CO_2$ saturated RSW cannot be used.

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# Production of the Euphausiid Crustacean *Thysanoëssa raschii* in the Gulf of St. Lawrence

F. BERKES[1]

*Marine Sciences Centre, McGill University, Montreal, Que.*

BERKES, F. 1977. Production of the euphausiid crustacean *Thysanoëssa raschii* in the Gulf of St. Lawrence. J. Fish. Res. Board Can. 34: 443–446.

From field data on growth and population numbers, production of *Thysanoëssa raschii*, excluding molts, was calculated to be about 1.8 mg dry wt·$m^{-3}$·$yr^{-1}$ in the Gulf of St. Lawrence. The mean annual biomass of *T. raschii* was 0.47 mg dry wt·$m^{-3}$, comprising about 1% of the total zooplankton biomass in the Gulf, and giving a production to biomass (P:B) ratio of about 4.

[1]Present address: Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1.

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Production and biomass were probably underestimated due to some sampler avoidance, lack of egg to nauplius–metanauplius interval of population production and loss of weight due to leaching in preserved samples.

BERKES, F. 1977. Production of the euphausiid crustacean *Thysanoëssa raschii* in the Gulf of St. Lawrence. J. Fish. Res. Board Can. 34: 443–446.

A partir de données cueillies sur le terrain sur la croissance et l'abondance des populations, on calcule que la production de *Thysanoëssa raschii*, mues non comprises, est d'environ 1.8 mg de poids sec·$m^{-3}$·$an^{-1}$ dans le golfe Saint-Laurent. La biomasse annuelle moyenne de *T. raschii* est de 0.47 mg de poids sec·$m^{-3}$, comprenant environ 1% de la biomasse zooplanctonique totale du Golfe, et donnant un rapport production:biomasse (P:B) d'environ 4. La production et la biomasse sont probablement sous-estimées à cause de l'évitement de l'échantillonneur, du manque de production de population dans l'intervalle de l'œuf au stade nauplius–métanauplius et de la perte de poids par lessivage dans les échantillons préservés.

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EUPHAUSIIDS are a key link between the smaller plankton and fish, and the assessment of euphausiid production is important for the study of resource potential of a marine area. Considerable data exist on fish production and on primary production, but as Steele (1974) pointed out, for the intermediate trophic levels knowledge is often limited to biomass.

Complex interplays between trophic levels, branching of food chains, and difficulties in determining food chain efficiencies (Steele 1974) introduce many uncertainties into the indirect estimation of zooplankton production. Direct estimates can be produced by ecological energetics or carbon budget studies (eg. Lasker 1966) or alternatively, by the use of field data on numbers and biomass for a series of time intervals over the life cycle of a species. The present study uses the latter approach and constitutes the first investigation of zooplankton production in the Gulf of St. Lawrence, based on population data on the euphausiid, *Thysanoëssa raschii*.

*Materials and methods* — The study is based on data from a total of 202 sampling stations in the Gulf at which there was at least one oblique tow from near bottom to surface. About two thirds of all samples were taken with standard 0.5-m nylon nets of no. 6 mesh (0.24 mm) and the rest with no. 0 mesh (0.57 mm) towed at about 1 m/s. All nets were metered with a TSK-type flow meter. More detail on sampling, areas, references to physicochemical data reports may be found in Berkes (1976) and Bulleid and Steven (1972).

Production was calculated directly from growth and population data, by tracing cohorts through time and integrating numbers and average weights to give production (see Mann 1969). Production (P) is expressed as

$$P = \sum_{i+1}^{S} (n_i - n_{i+1}) \left( \frac{\overline{w}_i + \overline{w}_{i+1}}{2} \right)$$

where S is the total number of time intervals used, $n_i$ is the population density of a certain year-class in numbers per unit volume of sea water, and $\overline{w}_i$ is the mean weight of an individual of that year-class. Thus, the production calculated between successive sampling dates represents tha' segment of the population $(n_i - n_{i+1})$ produced for the consumption of other trophic levels, of weights ranging from $\overline{w}_i$ to $\overline{w}_{i+1}$ during that time interval.

Population density of the larvae was based on smaller-mesh nets only and estimated indirectly from the ratio of adult *T. raschii* to *T. inermis* for spring cruises (May, June, and June–July). The younger larvae of the two species cannot be distinguished reliably (Einarsson 1945) and the two species have a similar brood size (Mauchline and Fisher 1969). Population density of the older *T. raschii* was established by counting all animals of a year-class in oblique (both mesh sizes), metered hauls in a given cruise and dividing this number by the total amount of water filtered, based on the assumption that the species was represented by one population in the Gulf. Data were treated as if they had come from one year-class sampled successively over the entire life cycle.

Population data for August over 2 yr were averaged for Group 0 and Group I. As Group 0 in the fall months appeared to be particularly "patchy" in distribution, with a small number of stations yielding high population numbers, the results of September and November cruises were also averaged. Egg numbers were not available, and the first three population numbers in production calculations represent a) a mixed population of nauplii and metanauplii larvae, b) Calyptopis II larvae of 1.5 mm avg length, and c) Furcilia II larvae of 3.2 mm avg length, respectively.

Weights for both biomass and production estimates were calculated on the basis of weight–length relation-

TABLE 1. Abundance of different year-classes of *Thysanoëssa raschii* in numbers per 100 m³. Each number represents the arithmetic mean of individuals of a year-class in all oblique tows in a given cruise.

| Cruise | Group 0 | Group I | Group II |
|---|---|---|---|
| Apr.–May 1970 | ? | 6.68 | 2.59 |
| May 1969 | 733.56 | 3.24 | 0.48 |
| June 1969 | 252.47 | 1.22 | 0.17 |
| June–July 1969 | 105.08 | 1.36 | |
| Aug. 1969 | 26.98 | 2.16 | |
| Aug. 1970 | 36.31 | 5.10 | |
| Sept. 1969 | 8.06 | 0.58 | |
| Nov. 1969 | 42.28 | 2.68 | |

ships given in Mauchline and Fisher (1969, p. 289), converted from wet to dry weights. These weight estimates were verified and supplemented by additional data, especially for smaller larvae, by the method of Lovegrove (1966).

*Results* — Table 1 gives the abundance of different year-classes of *T. raschii*. The youngest *T. raschii* sampled, nauplius–metanauplius stage larvae in May, were estimated at about 734 individuals/100 m³ over the entire Gulf, all depths. Survivorship to subsequent larval stages seemed to be low, population density decreasing to 105 individuals/100 m³ in June–July and eventually to 6.7/100 m³ by the following April–May, at which time the year-class became Group I. The rate of mortality appeared to decrease in the older *T. raschii*; Group II was 2.6/100 m³ the following April–May.

Table 2 summarizes the production of various intervals from population density and mean

TABLE 2. Population density, $n$ (no·m$^{-3}$), mean weight, $\bar{w}$ (milligrams dry weight), and production, P (milligrams·100 m$^{-3}$) of *T. raschii*.

| | | |
|---|---|---|
| $n_1$ = 733.56 | $\bar{w}_1$ = 0.0023 | $P_1$ = 6.30 |
| $n_2$ = 252.47 | $\bar{w}_2$ = 0.0239 | $P_2$ = 5.08 |
| $n_3$ = 105.08 | $\bar{w}_3$ = 0.045 | $P_3$ = 42.04 |
| $n_4$ = 31.64 | $\bar{w}_4$ = 1.1 | $P_4$ = 10.03 |
| $n_5$ = 25.17 | $\bar{w}_5$ = 2.1 | $P_5$ = 59.17 |
| $n_6$ = 6.68 | $\bar{w}_6$ = 4.3 | $P_6$ = 15.82 |
| $n_7$ = 3.24 | $\bar{w}_7$ = 4.9 | $P_7$ = 11.51 |
| $n_8$ = 1.22 | $\bar{w}_8$ = 6.5 | $P_8$ = −1.03 |
| $n_9$ = 1.36 | $\bar{w}_9$ = 8.2 | $P_9$ = −21.22 |
| $n_{10}$ = 3.63 | $\bar{w}_{10}$ = 10.5 | $P_{10}$ = 33.70 |
| $n_{11}$ = 0.58 | $\bar{w}_{11}$ = 11.6 | $P_{11}$ = −25.30 |
| $n_{12}$ = 2.68 | $\bar{w}_{12}$ = 12.5 | $P_{12}$ = 1.24 |
| $n_{13}$ = 2.59 | $\bar{w}_{13}$ = 15.0 | $P_{13}$ = 31.65 |
| $n_{14}$ = 0.48 | $\bar{w}_{14}$ = 15.0 | $P_{14}$ = 4.65 |
| $n_{15}$ = 0.17 | $\bar{w}_{15}$ = 15.0 | $P_{15}$ = 2.55 |
| $n_{16}$ = 0 | $\bar{w}_{16}$ = 15.0 | |
| | | Total P = 176.19 |

weight data. Population densities were calculated from Table 1 with the assumptions described in the methods section. Production of *T. raschii* amounted to about 4.8 mg dry wt·m$^{-3}$·yr$^{-1}$. In terms of carbon fixed per unit area, this was about 120 mg C·m$^{-2}$·yr$^{-1}$ if the volume of the Gulf is 34,550 km³ (Forrester 1964), the area 214,000 km² (Forrester and Vandall 1968), and if carbon is 42% of dry weight (Lasker 1966).

Group 0 accounted for about 60% of the total population production; the rest was almost equally divided between the two larger size-classes. The greatest production occurred in Group 0 between the June–July and the August sampling periods. Seasonal pulses of production are reflected to some degree also in Fig. 1 which gives the biomass or standing stocks of euphausiids for each cruise. The total zooplankton biomass (Bulleid and Steven 1972) is included for comparison. August biomass values tend to be minimal and the spring maximal. Over the sampling period, *T. raschii* was about 1% of total zooplankton biomass and about 17% of total euphausiid biomass (0.24-mm mesh nets only). The mean annual biomass of *T. raschii* was 0.47 mg dry wt·m$^{-3}$, giving a production to biomass ratio (P:B) of about 4.

*Discussion* — While the choice of *T. raschii* for production calculations probably minimized the problem of sampler avoidance, comparisons of different net sizes (see Jerde 1967) indicate that some avoidance probably did occur, leading to an underestimate of both the production and the biomass. Another source of error leading to underestimation is the absence of the egg to nauplius interval in the calculations. *Thysanoëssa raschii* in the Gulf starts to spawn in April–May (Berkes 1976) but the earliest sample is for mixed nauplius–metanauplius stage larvae in mid-May. The magnitude of the error thus introduced

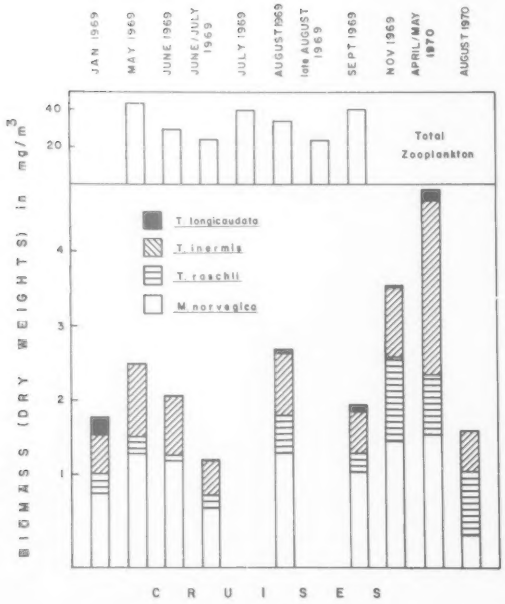


FIG. 1. Biomass of euphausiids and total zooplankton in the Gulf of St. Lawrence. Total zooplankton from Bulleid and Steven (1972).

may be estimated from the work of Lasker (1966) who calculated that egg production was 9% of assimilated carbon in *Euphausia pacifica*, assuming a 20-mo life span and a total fecundity of 1,400 eggs. A further source of error is the loss of weight due to leaching in preserved samples (Lovegrove 1966).

Since euphausiid generations are overlapping, the age may only be inferred from length-frequency distributions. Growth rates are much reduced in adults; thus, the age of the oldest *T. raschii* may only be guessed (See figure 5B, Berkes 1976). Ideally, production estimates may be based on a single-year class sampled over its entire life cycle; year-to-year variations in age-class strength, as suggested by Table 1, provide additional uncertainties for the calculations. Given these inaccuracies inherent in the estimates, further mathematical analyses, such as separating modes of size-groups or integration of the area under a numbers vs. weight curve (Mann 1969) were not attempted.

This minimal production estimate for *T. raschii*, 0.32 mg $C \cdot m^{-2} \cdot day^{-1}$, may be compared to that of the dominant euphausiid off the California coast, *E. pacifica*, 0.9 mg $C \cdot m^{-2} \cdot day^{-1}$, as calculated by Mullin (1969) from Lasker's (1966) carbon budget study. The ratio of 4 for annual production to mean biomass (P:B) of *T. raschii* appears higher than the value, 3.0, for *E. pacifica* (Lasker 1966) and 1.8 for *E. superba* as estimated by Allen (1971).

Production of euphausiids calculated by the population method, even with the above qualifications, still underestimates the total contribution of euphausiids to the energy flow in the food web. High frequency of molting, continuing through life (see Sameoto 1976), provides a major source of particulate matter in the ocean which is not accounted for by a calculation based on numbers and weight.

*Acknowledgments* — I thank Profs M. J. Dunbar and D. C. Maclellan, both of Marine Sciences Centre, McGill University, for stimulus, criticism, and assistance. The study is dedicated to the late Dr D. M. Steven, director of the IBP project, whose concern with plankton production in the Gulf inspired the present work. The study was supported by the National Research Council of Canada.

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## BOOK REVIEWS/COMPTES RENDUS

**Eel Capture, Culture, Processing and Marketing.** *D. M. Forrest. Fishing News Books Ltd., Fernham, Surrey, 1976. 195 p. £7.25.*

In his "Introduction" the author states, "Previous books have tended to describe only one or two aspects relating to eels, without bringing them all together under one cover. This is what this book does; . . ." and "In this book I have brought together all the relevant information published on eels." Although the book does not live up to these ambitious aims, it does provide a wealth of information on the culture of elvers and adult eels and will be invaluable for anyone intending to set up a pilot operation to determine the feasibility of eel culture.

The author has had the unique opportunity to travel to several countries involved in, or contemplating, eel culture. His visits to Japan and Taiwan, in which eel culture are carried out in a very intensive and competitive manner, have provided him with a solid base of practical experience which accounts for the authoritative way he tackles this subject. Much of his personal contact with the eel culture industry has been captured on film, and the book contains 66 photographs. While a few of these might have been omitted without any serious loss of understanding or interest by the reader, many of them do serve to illustrate technical points or serve to indicate the scope of an operation. The text is further illustrated by 24 line figures showing mainly layouts of culture ponds or tanks or flow charts. In some cases, the former are difficult to follow and a more extensive figure legend might have helped. None of the figures were referred to in the text. However, their proximity to the relevant text prevented confusion in most cases.

The book is divided into five sections: Elver capture, holding, and marketing; Eeel culture; Eel capture; Eel processing; Eel marketing. Almost 60% of the written text is devoted to the first two sections. The section on Eel culture, which included subsections on eel diseases, diet, eel-pond ecology, pond design and security, is written not only to provide information on methods used in other countries, but to provide a reasonable basis for establishing eel farms in countries (such as Canada) where eel culture is not practised. As the author points out, there are many practical and financial questions that need to be asked in a feasibility study. He lists several specific points that should be considered even before embarking on a pilot project. The author makes the point that he is writing as a layman for the layman. In general, this is true, but in some instances, as in the consideration of pH, the layman may be lost temporarily. In other instances, I felt the author might have been able to provide even more useful information than he chose to publish. For example, disease is a major factor in any animal culture operation. While a considerable body of information is presented on eel diseases, the author claims he leads the reader to where more technical information can be found on the subject. However, no references are referred to in the text and it is impossible to determine where further information can be found. A list of references is provided at the end of the book, but is rather a restricted list.

The section on Eel capture does not contain a great deal of new information as there have been other reviews on this topic. The methods the author describes relate mainly to European eels. No mention is made of the methods used to capture North American eels.

Some interesting and detailed technical information is provided in the section on Eel processing. However, some procedures, such as production of jellied eels, are restricted to such small geographic localities, and are of such limited general interest, that one queries their inclusion in such detail. Also, the listing by title of 57 varieties of eel dishes from Larousse Gastronomique seems somewhat out of place.

The section on marketing is based largely on FAO statistics from 1969–74.

Very little is included in this book on the life history and biology of eels and the book will be of little interest to the general biologist. However, it will be most useful to anyone wishing to hold eels for experimental purpose or wishing to engage in eel culture.

J. G. Eales

*Department of Zoology*
*University of Manitoba*
*Winnipeg, Man. R3T 2N2* (R118)

### Books Received/Livres Reçus

*August 1976*

**Identification and Analysis of Organic Pollutants in Water.** *Edited by Lawrence H. Keith. Ann Arbor Science Publishers Inc., Ann Arbor, Mich. 48106, 1976. xi + 718 p. $27.50.* (The material for this book comes largely from a symposium held at the First Chemical Congress of the North American Continent, Mexico City, December 1975.)

**Energy Flow — Its Biological Dimensions.** *Edited by Thomas W. M. Cameron and L. W. Billingsley.*

*Royal Society of Canada, Ottawa, K1A 0N4, 1975. x + 319 p. $5.00.* (A readable summary of Canadian achievements during participation in the International Biological Programme (IBP) from 1964 to 1974. Written for laymen who are interested in the flow of energy from the sun or other primary sources to plants and from them to animals on land, in fresh water, and in the sea.)

**Environmental Biogeochemistry.** *Edited by Jerome O. Nriagu. Vol. 1. Carbon, Nitrogen, Phosphorus, Sulfur and Selenium Cycles. Vol. 2. Metals Transfer and Ecological Mass Balances. Ann Arbor Science Publishers Inc., Ann Arbor, Mich. 48106, 1976. Vol. 1: xv + 1–423. Vol. 2: xv + 427–797 p. $29.50*

**Encyclopaedic Dictionary of Mathematics for Engineers and Applied Scientists.** *I. N. Sneddon. Pergamon Press, Ltd., Oxford, OX3 OBW. 1976. viii + 800 p. $100.* (A book to fill the need for easy reference to mathematical definitions and theories that is felt by biological and physical scientists in recent computer-oriented times.)

**Herbicides. Chemistry, Degradation and Mode of Action.** *Second Edition. Vol. 2. Edited by P. C. Kearney and D. D. Kaufman. Marcel Dekker, Inc., New York, 1976. xi + 1036 p. $37.50.* (Chapters on eight groups of herbicides plus chapters on photodecomposition and volatility have been written by 13 American, British and Japanese authors.)

**An Annotated Bibliography on the Pinnipedia.** *K. Ronald, L. M. Hanly, P. J. Healey, and L. J. Selley. International Council for the Exploration of the Sea, DK 2920 Charlottenlund, Denmark, 1976. x + 785 p.* (Consists of approximately 9500 references on seals, sea lions, and walruses. It is composed of two parts, the author index and the subject index.)

## COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN


Reductions in biomass and diversity resulting from exposure to mercury in artificial streams
**C. F. Sigmon, H. J. Kania, and R. J. Beyers**

Protective effect of chloride on nitrite toxicity to coho salmon (*Oncorhynchus kisutch*)
**S. J. Perrone and T. L. Meade**

Effects of bovine growth hormone on growth of carp (*Cyprinus carpio*) and the influences of temperature and photoperiod
**I. R. Adelman**

Evaluation of erythrocyte δ-amino levulinic acid dehydratase activity as a short-term indicator in fish of a harmful exposure to lead
**P. V. Hodson, B. R. Blunt, D. J. Spry, and K. Austen**

Biological utilization and regeneration of silicon in Lake Michigan
**H. L. Conway, J. I. Parker, E. M. Yaguchi, and D. L. Mellinger**

Dissolution of diatom frustules and recycling of amorphous silicon in Lake Michigan
**J. I. Parker, H. L. Conway, and E. M. Yaguchi**

Seasonal periodicity of diatoms, and silicon limitation in offshore Lake Michigan, 1975
**J. I. Parker, H. L. Conway, and E. M. Yaguchi**

Oceanographic features, currents, and transport in Cabot Strait
**M. I. El-Sabh**

Development of a blood sugar bioassay for rapidly measuring stressful levels of pulpmill effluent to salmonid fish
**D. J. McLeay**

Effects of thermal shocks on drifting aquatic insects: a laboratory simulation
**F. F. Sherberger, E. F. Benfield, K. L. Dickson, and J. Cairns Jr.**

The flathead sole (*Hippoglossoides*) in the North Pacific
**C. R. Forrester, H. Tsuyuki, S. Fuke, J. E. Smith, and J. Schnute**

On the variability and taxonomy of *Pleuroxus denticulatus* Birge (Cladocera: Chydoridae)
**D. Flössner and K. Kraus**


Notes


Avoidance of bleached kraft pulpmill effluent by pinfish (*Lagodon rhomboides*) and gulf killifish (*Fundulus grandis*)
**F. G. Lewis III and R. J. Livingston**

Efficiency of nets and a new device for sampling living fish larvae
**P. M. McGroddy and R. L. Wyman**

A colorimetric method for determination of saxitoxin
**R. M. Gershey, R. A. Nevé, D. L. Musgrave, and P. B. Reichardt**

Effect of meiofauna on incorporation of aged eelgrass, *Zostera marina*, detritus by the polychaete *Nephthys incisa*
**K. R. Tenore, J. H. Teitjen, and J. J. Lee**

Egg hatchability and tolerance of brook trout (*Salvelinus fontinalis*) fry at low pH
**J. R. Trojnar**

Letters/Lettres: JPU theory and the apotheosis of simulacra
**S. R. Kerr,** Editor's Note, **J. C. Stevenson**

# RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

## Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

2263. MILLER, R. J. 1976. North American crab fisheries: Regulations and their rationales. Fish. Bull. 74: 623–633. (9)

2264. SCOTT, K. R. 1976. Hot water thawing of deboned mullet blocks. J. Can. Inst. Food Sci. Technol. 9: 160–166 (4)

2265. GILGAN, M. W., T. E. FARQUHARSON, AND G. B. BURNS. 1977. The effect of a-ecdysone, ecdysterone and inokosterone treatment, separately or in combinations, on premolt development and molting in adult male lobsters, *Homarus americanus*. Comp. Biochem. Physiol. A 56: 43–49. (8)

2266. ZITKO, V. 1976. Levels of chlorinated hydrocarbons in eggs of doubled-crested cormorants from 1971 to 1975. Bull. Environ. Contam. Toxicol. 16: 399–405. (6)

2267. SHIEH, H. S. 1976. Effect of aeromonas salmonicida *Furunculosis* infection on the incorporation of (1-$^{14}$C) acetate into lipids of trout liver. Infect. Immun. 14: 836–838. (8)

2268. BERLAND, B. R., D. J. BONIN, S. Y. MAESTRINI, M. L. LIZARRAGA-PARTIDA, AND N. J. ANTIA. 1976. The nitrogen concentration requirement of D-glucosamine for supporting effective growth of marine microalgae. J. Mar. Biol. Assoc. U.K. 56: 629–637. (3)

2269. WHYTE, J. N. C., AND J. R. ENGLAR. 1976. Determination of halogens in marine algae by use of an ion-selective electrode. Analyst 101: 815–819. (2)

2270. MOUNIB, M. S., H. ROSENTHAL, AND J. S. EISAN. 1976. Effects of cadmium on developing eggs of the Pacific herring with particular reference to carbon dioxide fixing enzymes. Biol. Reprod. 15: 423–428. (8)

2271. SMITH, T. G. 1976. Predation of ringed seal pups *Phoca hispida* by the arctic fox *Alopex lagopus*. Can. J. Zool. 54: 1610–1616. (5)

2272. SMITH, T. G. 1975. Ringed seals in James Bay and Hudson Bay: Population estimates and catch statistics. Arctic 28: 170–182. (5)

2273. WALLACE, R. R., H. B. N. HYNES, AND W. F. MERRITT. 1976. Laboratory and field experiments with methoxychlor as a larvicide for simuliidae *Diptera*. Environ. Pollut. 10: 251–269. (4)

## Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

## Interpretive Articles Series

This new series commenced in 1966. The articles interpret work already published in primary journals. They appear at irregular intervals in various technical publications. Frequently an article essentially duplicates, condenses, or is based on an article in another series.

346. SMITH, T. G. 1976. Biology of the Beaufort Sea. Appendix 4. In oil under the ice. Can. Arctic Res. Comm. p. 147–152. (5)

347. SERGEANT, D. E. 1976. History and present status of populations of harp and hooded seals. Biol. Conserv. 10: 95–118. (5)

348. BECK, B., AND T. G. SMITH. 1976. Seal finger: an unsolved medical problem in Canada. Can. Med. Assoc. J. 17: 105. (5)

349. MACASKIE, I. B. 1975. A third sea otter transplant to British Columbia. The Beaver. Spring: 9–11. (5)

## Articles explicatifs

Cette série a débuté en 1966. Les articles donnent une explication de travaux déjà publiés dans des revues prioritaires. Ils paraissent à intervalles irréguliers, dans diverses publications techniques. Souvent, un article se basera sur un écrit d'une autre série, le condensera ou le reproduira presque tel quel.

## Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 22¢ per page and a minimum charge of $2.20. Information on translations can be obtained from the Scientific Information and Publications Branch.

DUDA, V. I., AND S. V. DOBRITSA. 1976. Changes in the nucleotide composition of DNA in *Clostridium pectinofermentans* depending on the stage of development of a culture. Dokl. Akad. Nauk SSSR 227: 477–480. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3864, 1976)

FILATOVA, Z. A. 1948. Class: Bivalvia, Mollusca, p. 405–446. *In* Keys to the fauna and flora of the Northern Seas of the USSR. State Publications, Moscow. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3865, 1976)

GUIDETTI, L. M., C. SARRA, M. BOCCIGNONE, AND L. LONGHI. 1975. Toxic and dystrophyic manifestations in rats on a diet with a high salted lard content. Minerva Dietol. (15): 57–59. (Transl. from Italian by Fish. Mar. Serv. Transl. Ser. No. 3866, 1976)

BOEV, A. A., R. N. STEPANOVA, AND B. G. TRAVKIN. 1975. Biotechnology of hormone stimulation of the maturation of bream in relation to intensified bream rearing in the Volga Delta. Tr. Vses. Nauchno Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 111: 172–176. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3867, 1976)

LLUIS, C., AND J. BOZAL. 1976. Structural analogues of pyruvate. Behaviour as substrates or inhibitors of LDH. Rev. Esp. Fisiol. 32: 9–13. (Transl. from Spanish by Fish. Mar. Serv. Transl. Ser. No. 3868, 1976)

LE PETIT, L., AND M.-H. N'GUYEN. 1976. Phosphorus requirements for bacteria that metabolize hydrocarbons in the sea. Can. J. Microbiol. 22: 1364–1373. (Transl. from French by Fish. Mar. Serv. Transl. Ser. No. 3869, 1976)

KOCHI, M. 1976. Separation of isomeric eicosadienoic acid from sea-urchin lipid. Nippon Suisan Gakkaishi 42: 491–496. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3870, 1976)

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MOISEEVA, E. B. 1975. Histophysiology of hypothalamo-hypophysial system of some marine fish in relation to spawning types. Tr. Vses. Nauchno Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 111: 106–124. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3873, 1976)

## Traductions

Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 22¢ la page et à un prix minimum de $2.20 aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. La Direction de l'information et des publications scientifiques peut fournir des renseignements sur ces traductions.

KOCHI, M. 1976. Determination of double bond positions in isomeric eicosadienoic acids from sea-urchin lipid. Nippon Suisan Gakkaishi 42: 721–729. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3874, 1976)

KELLER, A. 1975. The drift and its ecological significance. Experimental investigation on *Ecdyonurus venosus* (FABR.) in a stream model. Schweiz. Z. Hydrol. 37: 294–331. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3875, 1976)

LANZOLA, E., C. ROGGI, A. MONTANI, AND M. ALLEGRINI. 1975. Metabolic toxicological studies on rats fed high rapeseed and peanut oil content diets for 90 days. Ig. Sanita Pubblica 31: 121–137. (Transl. from Italian by Fish. Mar. Serv. Transl. Ser. No. 3876, 1976)

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YASHNOV, V. A. 1948. Leptostraca, Mysidacea, Cumacea, Isopoda, Amphipoda, p. 233–257. *In* Keys to the fauna and flora of northern seas of the USSR. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3880, 1976)

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ASCIK, K., M. GLINSKA, AND K. SZYPRUC. 1975. Determination of toxic substances in paper mill industry. Przegl. Papier. 31: 232–233. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 3887, 1976)

KOELBEL, H. K. 1976. Carbon supporting films for high resolution electron microscopy: improvement of quality and preparation technique. Mikroskopie 32: 1–16. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3888, 1976)

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MARKIN, V. A. 1975. Some aspects of the theory and design of marine fish trap nets. (tables, figures and summary only) Izv. Tikhookean. Nauchno Issled. Inst. Rybn. Khoz. Okeanogr. 94: 94–118. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3891, 1976)

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SEHER, A., AND V. VOGEL. 1976. Studies on sterol mixtures V: Sterols in sunflower oil and other plant oils. Fette Seifen Anstrichmittel 78: 301–305. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3899, 1977)

## List of Establishments

(1) Department of Fisheries and the Environment
Fisheries and Marine Service
Pacific Biological Station
P.O. Drawer 100
Nanaimo, British Columbia V9R 5K6

(2) Department of Fisheries and the Environment
Fisheries and Marine Service
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver, British Columbia V6T 1X2

(3) Department of Fisheries and the Environment
Fisheries and Marine Service
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia V7V 1N6

(4) Department of Fisheries and the Environment
Fisheries and Marine Service
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba R3T 2N6

(5) Department of Fisheries and the Environment
Fisheries and Marine Service
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec H9X 3L6

(6) Department of Fisheries and the Environment
Fisheries and Marine Service
Biological Station
St. Andrews, New Brunswick E0G 2X0

(7) Department of Fisheries and the Environment
Fisheries and Marine Service
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia B2Y 4A2

(8) Department of Fisheries and the Environment
Fisheries and Marine Service
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia B3J 2R3

(9) Department of Fisheries and the Environment
Fisheries and Marine Service
Biological Station
St. John's, Newfoundland A1C 1A1

(10) Department of Fisheries and the Environment
Fisheries and Marine Service
Office of the Editor
116 Lisgar Street
Ottawa, Ontario K1A 0H3

(11) Department of Fisheries and the Environment
Fisheries and Marine Service
Canada Centre for Inland Waters
Great Lakes Biolimnology Laboratory
P.O. Box 5050
867 Lakeshore Rd.
Burlington, Ontario L7R 4A6

## Liste d'établissements

(1) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Station biologique du Pacifique
C.P. 100
Nanaïmo (Colombie-Britannique) V9R 5K6

(2) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Laboratoire de Vancouver
6640 nord-ouest, prom. Marine
Vancouver (Colombie-Britannique) V6T 1X2

(3) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Institut de l'environnement du Pacifique
4160, prom. Marine
Vancouver-Ouest (Colombie-Britannique)
V7V 1N6

(4) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Institut des eaux douces
501. University Crescent
Winnipeg (Manitoba) R3T 2N6

(5) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Station biologique de l'Arctique
C.P. 400
Sainte-Anne-de-Bellevue (Québec) H9X 3L6

(6) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Station biologique
St. Andrews (Nouveau-Brunswick) E0G 2X0

(7) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Laboratoire d'écologie marine
Institut de Bedford C.P. 1006
Dartmouth (Nouvelle-Ecosse) B2Y 4A2

(8) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Laboratoire d'Halifax
C.P. 429
Halifax (Nouvelle-Ecosse) B3J 2R3

(9) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Station biologique
Saint-Jean (Terre-Neuve) A1C 5X1

(10) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Bureau du rédacteur
116, rue Lisgar
Ottawa (Ontario) K1A 0H3

(11) Ministère des pêches et de l'Environnement
Service des pêches et des sciences de la mer
Centre canadien des eaux intérieures
Laboratoire de biolimnologie des Grands Lacs
C.P. 5050
867, ch. Lakeshore
Burlington (Ontario) L7R 4A6

## Annual Meeting of the American Society of Zoologists and Society of Systematic Zoology Hosted by the Canadian Society of Zoologists

The annual meeting of the American Society of Zoologists and the Society of Systematic Zoology hosted by the Canadian Society of Zoologists will be held December 27–30, 1977 in Toronto, Canada, at the Royal York Hotel.

The following symposia are planned: Comparative Heart Physiology, Developmental and Evolutionary Aspects of Brain Endocrine Function, Antigen Recognition, Comparative Tumor Immunology, Comparative Auto-Immune Disease Models, Vertebrate Appendage Regeneration, Polyamines in Embryonic Development, Neuromuscular Interactions, Marine Larvae: Metamorphosis and Settlement, Nectar Feeding in Birds, Adaptations to Adverse Environments, Physical Events that Affect Biotic Patterns, Evolutionary Mechanisms and Processes, and Phenetic and Phylogenetic Concepts and Methods of Classification.

A call for contributed papers will be issued in April 1977. For more details and forms write to: Ms Mary Wiley, American Society of Zoologists, Box 2739, California Lutheran College, Thousand Oaks, Calif. 91360, USA.

## Réunion annuelle de l'American Society of Zoologists et de la Society of Systematic Zoology sous les auspices de la Société canadienne des zoologistes

L'American Society of Zoologists et la Society of Systematic Zoology seront les invités de la Société canadienne des zoologistes lors de leur réunion annuelle qui se tiendra à l'hôtel Royal York, Toronto, Canada, les 27–30 décembre 1977.

On se propose d'organiser des colloques sur les sujets suivants : physiologie cardiaque comparative, développement et évolution de la fonction endocrine du cerveau, reconnaissance des antigènes, immunologie comparative des tumeurs, modèles comparatifs d'auto-immunité contre les maladies, régénération des membres chez les vertébrés, polyamines dans le développement embryonnaire, interactions neuromusculaires, larves marines : métamorphose et fixation, alimentation au nectar chez les oiseaux, adaptations à des environnements adverses, événements physiques affectant les schèmes biotiques, mécanismes et processus évolutifs, et concepts phénétiques et phylogéniques dans les méthodes de classification.

On fera appel d'articles en avril 1977. Pour détails supplémentaires et formulaires, s'adresser à : Ms Mary Wiley, American Society of Zoologists, Box 2739, California Lutheran College, Thousand Oaks, Calif. 91360, USA.

## English Translations of Polish Language Reports on Bacterial Hemorrhagic Septicemia

One of the most important disease problems of carp husbandry is bacterial hemorrhagic septicemia. Dr S. F. Snieszko notes, "Several important papers on this subject have appeared lately that have not taken into consideration the findings reported in these (early Polish language) papers." Acknowledging the requests of interested researchers, Dr Snieszko has translated the following papers, and they are available by writing: Librarian, Eastern Fish Disease Laboratory, Route 1, Box 17A, Kearneysville, West Virginia 25430, USA.


SNIESZKO, S. 1937. Wyniki badań nad bakterjami t. zw. posocznicy karpi. (Results of investigations on the so called septicemia of carp). Przeglad Rybacki 10(8): 309–311.

SNIESZKO, S., W. PIOTROWSKA, B. KOCYŁOWSKI, AND K. MAREK. 1938. Badania bakteriologiczne i serologiczne nad bakteriami posocznicy karpi. (German subtitle: Bakteriologische und Serologische Untersuchungen über die Bakterien der Karpfenseuche). (Bacteriological and serological studies on bacteria of the carp hemorrhagic septicemia). Rozprawy Biologiczne z Zakresu Medycyny Weterynaryjnej, Rolnictwa i Hodowli 16(1–2): 1–15.

SPICZAKOW, T. 1933. Posocznica Karpi (Choroba lubelska). Septicaemia haemorrhagica cyprinorum. (Hemorrhagic septicemia of carp). Przeglad Rybacki 6(8): 253–265.


## Traductions anglaises d'articles en polonais sur la septicémie hémorragique bactérienne

Une des plus importantes maladies posant des problèmes aux éleveurs de carpes est la septicémie hémorragique bactérienne. Selon M. S. F. Snieszko, « il a paru dernièrement plusieurs articles importants sur ce sujet qui ne tiennent pas compte des découvertes signalées dans des articles antérieurs en langue polonaise. » A la demande de chercheurs intéressés, M. Snieszko a traduit les articles suivants, que l'on peut se procurer en s'adressant à : Librarian, Eastern Fish Disease Laboratory, Route 1, Box 17A, Kearneysville, West Virginia 25430, USA.


SNIESZKO, S. 1937. Wyniki badań nad bakterjami t. zw. posocznicy karpi. (Results of investigations on the so called septicemia of carp). Przeglad Rybacki 10(8): 309–311. (Résultats de recherches sur la septicémie de la carpe)

SNIESZKO, S., W. PIOTROWSKA, B. KOCYŁOWSKI, AND K. MAREK. 1938. Badania bakteriologiczne i serologiczne nad bakteriami posocznicy karpi. (Sous-titre allemand : Bakteriologische und Serologische Untersuchungen über die Bakterien der Karpfenseuche). (Bacteriological and serological studies on bacteria of the carp hemorrhagic septicemia). Rozprawy Biologiczne z Zakresu Medycyny Weterynaryjnej, Rolnictwa i Hodowli 16(1–2): 1–15. (Etudes bactériologiques et sérologiques des bactéries de la septicémie hémorragique de la carpe)

SPICZAKOW, T. 1933. Posocznica Karpi (Choroba lubelska). Septicaemia haemorrhagica cyprinorum. (Hemorrhagic septicemia of carp). Przeglad Rybacki 6(8): 253–265. (Septicémie hémorragique de la carpe)

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### FISHERIES SOCIETY OF THE BRITISH ISLES

A Symposium will be held at the University of Stirling, from July 4–8, 1977 on: *Rhythmic Activity of Fish*

**Hormonal Controls**
**Behavioral Rhythms**
**Community Relationships**
**Methods and Instruments**

Contributors will include:

H. Braithwaite, University of Birmingham, England
L-O Eriksson, Max Planck Institute, Erling-Andechs, Germany
B. P. Manteifel, Servertsov Institute, Moscow, USSR
A. J. Matty, University of Aston, Birmingham, England
K. Muller, Royal Swedish Academy of Sciences, Abisko, Sweden
W. R. A. Muntz, University of Stirling, Scotland
I. G. Priede, University of Aberdeen, Scotland
H. O. Schwassmann, University of Florida, USA
D. Simpson, North of Scotland Hydro-Electric Board, Pitlochry, Scotland
T. H. Simpson, DAFS, Marine Laboratory, Aberdeen, Scotland
D. J. Staples, CSIRO, Karumba, Australia
T. Storeton-West, MAAF Marine Laboratory, Lowestoft, England

Further details of the program, bookings, etc., will be obtainable in October 1976 from the symposium organizer:

*J. E. Thorpe*
*Freshwater Fisheries Laboratory*
*Pitlochry*
*Perthshire, PH16 5LB*
*Scotland*

## PREAVIS

### FISHERIES SOCIETY OF THE BRITISH ISLES

Il se tiendra un colloque à l'Université de Stirling du 4 au 8 juillet 1977 sur: *L'activité ryhtmique des poissons*

**Contrôles hormonaux**
**Ryhtmes de comportement**
**Relations entre les communautés**
**Méthodes et instruments**

Parmi les contributeurs, il y aura:

H. Braithwaite, Université de Birmingham, Angleterre
L. O. Eriksson, Institut Max Planck, Erling-Andechs, Allemagne
B. P. Manteifel, Institut Servertsov, Moscou, URSS
A. J. Matty, Université d'Aston, Birmingham, Angleterre
K. Muller, Royal Swedish Academy of Sciences, Abisko, Suède
W. R. A. Muntz, Université de Stirling, Ecosse
I. G. Priede, Université d'Aberdeen, Ecosse
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T. H. Simpson, DAFS, Laboratoire maritime, Aberdeen, Ecosse
D. J. Staples, CSIRO, Karumba, Australie
T. Storeton-West, MAAF, Laboratoire maritime, Lowestoft, Angleterre

On pourra se procurer en octobre 1976 plus de détails sur le programme, les réservations, etc., en s'adressant à l'organisateur du colloque:

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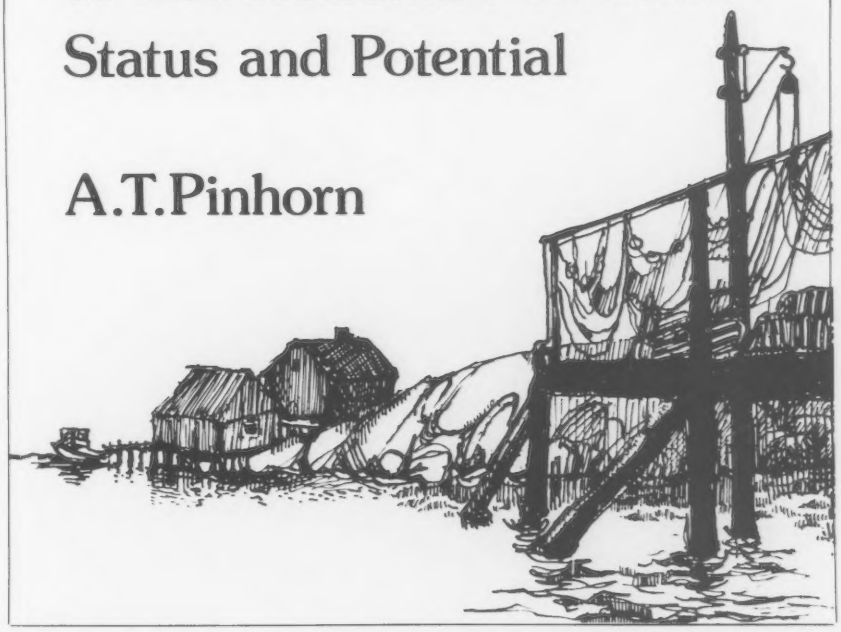
Bulletin of the Fisheries Research Board of Canada

Bulletin 194 Ottawa 1976

# Living Marine Resources of Newfoundland~Labrador: Status and Potential

A.T.Pinhorn



Knowledge on the marine fisheries resources of Newfoundland and Labrador to 1964 was summarized in Bulletin 154 of the Fisheries Research Board of Canada. Additional information, which has become available since that time, is summarized here. Scientific basis for regulating and managing the international fisheries in the Newfoundland and Labrador area through the International Commission for the Northwest Atlantic Fisheries (ICNAF) is also duscussed.

Current information is presented for all species that presently support or have potential for supporting commercial fisheries in the area. Detail given varies depending on the present extent of knowledge and previous coverage of the species. It is presented on a species-by-species basis and, when possible, on a stock-by-stock basis within species. Pertinent information is given on the distribution, abundance, general biology, and fishery of each species, especially where this was not previously covered.

Also, since in the late 1960s and 1970s regulations of one form or another have been introduced for many species, an extensive treatment of the management regime for such species is presented. Potential for increased catches from all these resources is summarized.

*(Canada: $4.00, Other countries: $4.80; Cat. No. Fs 94-194; 64 pages)*

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Journal de l'office des recherches sur les pêcheries du Canada

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